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Phytodepuration of contaminated effluents obtained in hydrothermal carbonization processes

Thesis submitted for the Degree of Master in Biotechnology

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Abstract

The scarcity of resources in recent decades has increased the search for new alternative and renewable forms of energy to fossil fuels. However, this transition must be carried out to achieve a more diversified energy system based on sustainability and environmental protection. In recent years, hydrothermal carbonization (HTC) has been considered an alternative process for processing value added products. This alternative method is a thermochemical process that converts high moisture organic feedstock into carbon rich solids. However, during this process toxic organic and inorganic compounds present in the feedstock, such as hydrocarbons, are leached to a liquid phase that needs to be depurated. Phytodepuration is a technology based on the use of plants in the remediation of contaminated effluents, thus it's a sustainable option that allows to simultaneously clean the contaminated waters obtained in the hydrothermal carbonization process and produce biomass that can be used in multiple applications. *Camelina sativa* is an energy crop that has the potential to produce biofuels, as well as, value added products from its oil. Several studies have shown that energy crops are tolerant to irrigation with contaminated waters. In this work we evaluated the phytodepuration capacity of *Camelina sativa* (winter and spring varieties) when subjected to the irrigation of contaminated effluents obtained in hydrothermal carbonization process, as well as, the productivity and quality of biomass. The hydrothermal carbonization effluent (HTC) used in this study (360 mg / L O_2) was diluted 1:3 (WW1: 120 mg / L O_2), 1:2.4 (WW2: 150 mg / L O_2) and 1:2 (WW3: 180 mg / L O_2) to obtain the chemical oxygen demand (CDO) equal to 0.8, 1.0 and 1.2 times the limit value for wastewater discharges established by Decree Law 236/98. Also, the pots were irrigated with tap water as a control treatment. The results obtained led to the conclusion that the soil-biomass system was able to depurate the contaminated waters from the HTC process, decreasing by *circa* 63-72% for WW1, 69-75% for WW2 and 68-76% for WW3 the initial oxidability values and thus, avoiding the contamination of groundwater. Regarding biomass productivity, it's was concluded that the winter variety of *Camelina sativa* was the least affected by the contaminated waters of the HTC effluent. In addition, the winter variety obtained the highest productivity, namely in the aboveground and siliquae yields (loss of siliquae yield: 9-45% for the winter variety; 11-67% for the spring variety). Ash, nitrogen, metals and phosphorus content in the plant were also affected by the contaminated effluents from the HTC process.

Keywords: *Hydrothermal carbonization (HTC); Sustainability; Energy crops; Contaminated effluents remediation; Phytodepuration; Camelina sativa*

Resumo

A escassez de recursos nas últimas décadas potenciou a procura de novas formas de energia alternativas e renováveis aos combustíveis fósseis. No entanto, esta transição deve ser realizada com a finalidade de se obter um sistema energético mais diversificado tendo como base a sustentabilidade e a proteção do ambiente. Nos últimos anos, a carbonização hidrotérmica (HTC) tem sido considerada um processo alternativo de processamento de produtos de valor acrescentado. Este método alternativo é um processo termoquímico que converte matéria-prima orgânica de elevada humidade em produtos sólidos ricos em carbono. No entanto, durante este processo compostos orgânicos e inorgânicos tóxicos presentes na biomassa bruta, como por exemplo hidrocarbonetos, são lixiviados produzindo uma fase líquida que é necessário depurar. A fitodepuração é uma tecnologia que tem como base a utilização de plantas na remediação de efluentes contaminados, apresentando-se assim, como uma opção sustentável que permite simultaneamente tratar as águas contaminadas obtidas no processo de carbonização hidrotérmica e produzir biomassa que pode ser utilizada em múltiplas aplicações. *Camelina sativa* é uma cultura energética que tem a potencialidade de produzir biocombustíveis, bem como produtos de valor acrescentado a partir do seu óleo. Diversos estudos comprovam que as culturas energéticas são tolerantes à rega com águas contaminadas. Neste trabalho foi avaliada a capacidade fitodepuradora de *Camelina sativa* (variante de inverno e primavera) quando sujeita à rega com efluentes contaminados obtidos em processo de carbonização hidrotérmica, bem como a produtividade e qualidade da biomassa obtida. O efluente de carbonização hidrotérmica (HTC) utilizado neste estudo foi diluído (360 mg/L O_2) de 1:3 (AR1: 120 mg/L O_2), 1:2,4 (AR2:150mg/L O_2) e 1:2 (Ar3: 180 mg/L O2) para se obter o valor limite de carência química de oxigénio (CQO) igual a 0.8,1 e 1.2 vezes o limite para descargas de águas residuais estabelecido pelo Decreto de Lei 236/98. Para além disso, os vasos foram regados com água da torneira como controlo. Os resultados obtidos permitiram concluir que o sistema solo-biomassa conseguiu depurar as águas contaminadas no processo HTC, evitando assim, a contaminação de lençóis freáticos. Os resultados das águas de percolação verificaram que o sistema solo-biomassa conseguiu depurar o efluente de HTC, uma vez que os valores de oxidabilidade diminuíram aproximadamente 63-72% para AR1, 69-75% para AR2 e 68-76% para AR3. Em relação à produtividade da biomassa, conclui-se que a variante de inverno de *C. sativa* foi a menos afetada pelas águas contaminadas do processo HTC. Para além disso, esta variante apresenta a maior produtividade, nomeadamente na produtividade aérea global e de vagens (perda de produtividade de siliquae: 9-45% para a variante de inverno;11-67% para a variante de primavera). O conteúdo de cinzas, metais, azoto e de fósforo na planta foram igualmente afetados pelas águas contaminadas do processo HTC.

Palavras-chave: *Carbonização hidrotérmica (HTC); Sustentabilidade; Cultura energética; Remediação de efluentes contaminados; Fitodepuração; Camelina sativa*

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List of Abbreviations

BOD_5 -Biochemical oxygen demand

COD-Chemical oxygen demand

DDT-Diclorodiphenyltrichloroethane
DLLME-Dispersive liquid liquid microextraction

GC/MS-Gas chromatography mass spectrometry

GHG-Greenhouse gases

HTC-Hydrothermal carbonization

IEO-International energy outlook

PCU-Platin cobalt units

RDF-Refuse derived fuels

Rpm- Rotation per minute

UV-Ultraviolet radiation

ELV-Emission limit value

MRV-Maximum recommended value

MPV-Maximum permissible value

WW1-Wastewater 1

WW2-Wastewater2

WW3-Wastewater 3

Chemical Formulas

C_6H_5OH-Phenol

Ca-Calcium

$CaCO_3$-Calcium carbonate

C-Carbon

Cd-Cadmium

CH_4- Methane

CO_2- Carbon dioxide

Cr-Chromium

Cu-Copper

Fe-Iron

H_2SO_4-Sulfuric acid

H_3O^+ - Acidic hydronium ions

HC-Hydrocarbons

H-Hydrogen

K_2SO_4-Potassium sulfate

K-Potassium

Mg-Magnesium

Mn-Manganese

N_2O-Nitrous oxide

Na-Sodium

NH_3-Ammonia

NH_4NO_3-Ammonium nitrate

Ni-Nickel

N-Nitrogen

NO-Nitrogen monoxide

NO_x-Nitrogen oxides

OH^- -Hydroxide ions

O-Oxygen

P_2O_5-Potassium oxide V

Pb-Lead

P-Phosphor

S-Sulfur

Zn-Zinc

Units of measure

µl-Microliter

µm-Micrometer

g-Gram

g/L-Gram per liter

g/m^2-Gram per square meter

Kg-Kilogram

mg/L-Milligram per liter

ml-Milliliter

mm-Millimeter

mS/cm-Millisiemens per centimeter

nm-Nanometer

^{o}C-Degrees celsius

1. The importance of the alternatives to fossil fuels

Since the beginning of the industrial revolution in the late 18th and early 19th century, energy has become an indispensable element for humanity's everyday life as well as for global economic growth. (Atabani, *et al*.,2012) The energy can be categorized in two main groups: renewable energy and non-renewable energy. The first type of energy are the clean sources of energy that are available in nature, such as wind, solar, hydroelectric, wave and bioenergy such as biomass and biofuel. Non-Renewable energy is based on coal, crude oil, natural gas which represents 80% of the total energy demand worldwide. (Koh *et al*.,2008). Figure 1.1 shows a schematic representation of the various types of energy present today globally

This growing demand for non-renewable energy sources, such as gasoline and diesel fuel are mainly due to the large transportation, electrical, and manufacturing industries. (Rizwanul *et al*.,2013). Of these industries, the transport industry will contribute about 63% to the increase in total global consumption of liquid fuels between 2010-2040 (IEO 2040; June 2013).

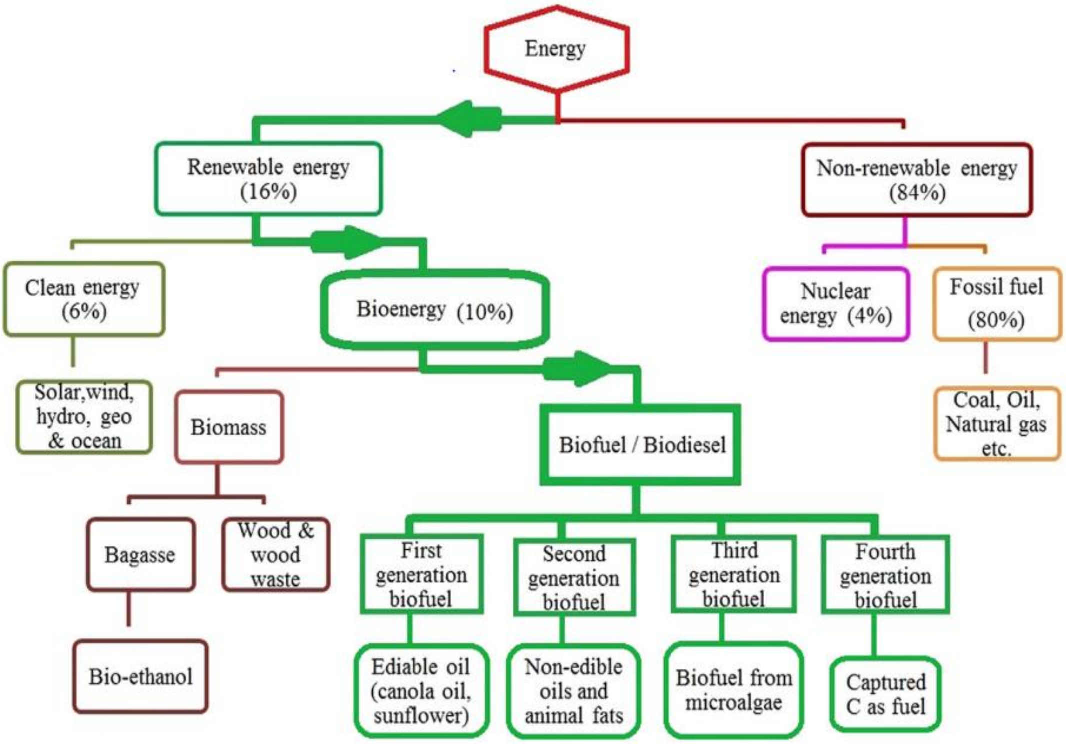

Figure 1.1- Classification of the various types of energy in the market. (Azad *et al*.,2015)

The reserves of coal, oil and natural gas are scarce globally which consequently increases the price of this fossil fuels (Derminbas., 2007). In addition, these fossil fuels are highly polluting, contributing to the emission of pollutants to the planet. It's estimated that about 22% of global emissions of greenhouse gases (GHGs) come only from the transport sector. These vehicles emit a diversity of pollutants such as carbon monoxide (CO), carbon dioxide (CO_2), nitrogen oxides (NO_x) and hydrocarbons (HC) that contribute for the degradation of air quality (Mofijur *et al*., 2016) (IEO,2013).

In this way, it's necessary to find alternatives to non-renewable energies based on sustainability, reduction of GHG, cost effective, freely available in nature, renewable and friendly for environment (Azad *et al*.,2015). One of the solutions to these environmental and economic issues is biofuel, as it presents itself as a renewable, sustainable and efficient energy. Biofuels are fuels derived from biomass and can be solid fuels, liquid fuels and diverse biogases (Janda *et al*., 2012). There are a wide variety of biofuels such as bioethanol which is gasoline equivalent, biodiesel which is diesel equivalent, biobutanol, biohydrogen and biodiesel biogas (Kapasi *et al*.,2010) (Antizar-Ladislao *et al*.,2008). Figure 1.2 shows the various possibilities of biodiesel as a substitute for diesel.

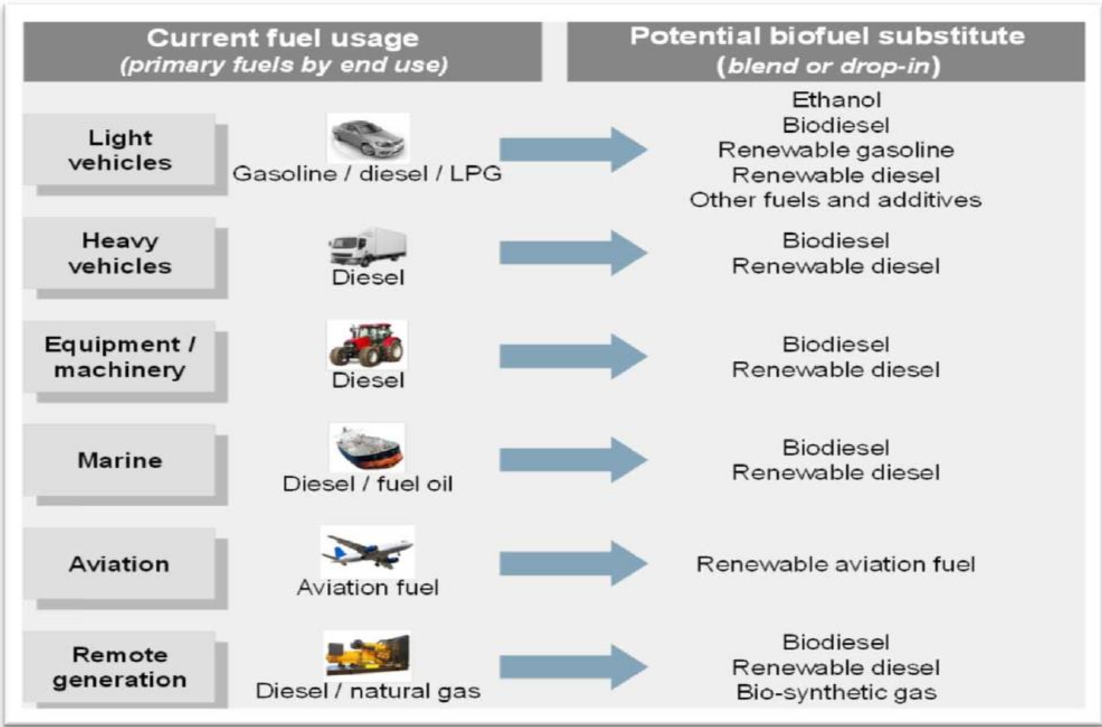

Figure 1.2- Diverse possibilities for biodiesel as a substitute for diesel. (Advanced Biofuels Study Strategic Directions for Australia. L.E.K.; 2011)

There are four generations of biofuels of which 2G,3G and 4G are considered advanced biofuels (Azad *et al*.,2015). The first generation (1G) has as sources food crops, sugar, starch, animal or oil fats, for instance 1G bioethanol is produce using soybean oil as feedstock. These 1G biofuels have numerous problems such as socio and economic because they derived from food crop feedstocks which consequently increases food price and uses agricultural land (Azad *et al*.,2013). The second generation (2G) biofuels are based on non-food crops such as municipal solid waste, stalks of wheat and waste cooking oil. This generation produce biohydrogen, biomethanol and mixed alcohols which don´t have socio and economic problems (Dermirbas., 2009). The third generation (3G) biofuels are from algae biomass which have great advantages such as low-cost cultivation, high energy, eco-friendly and entirely renewable feedstock (Lee RA *et al*., 2013). Finally, the fourth generation (4G) has biofuels produced from captured carbon from the hydro-processing, advanced biochemistry, geosynthesis or low pressure and low temperature electrochemical processes (Lu *et al*., 2011). Table 1.1 presents the different feedstocks of each generation of biofuels and their products.

In addition to the above, there is the production of bioenergy by thermochemical processes such as combustion, gasification, pyrolysis, liquefaction and hydrothermal upgrading. These processes are characterized by the supply of heat in the presence or absence of an oxidizing agent and are intended primarily for low moisture feedstocks. The major advantages of these processes are the short reaction time and high conversion efficiency (Ahmed *et al.*, 2018).

Table 1.1-Representation of the different feedstocks of each generation of biofuels and their products. (Adapted, Dermirbas., 2009)

Generation	Feedstocks	Example of products
1G biofuel	Rice, wheat, sugar, edible vegetable, etc.	Bioethanol, biodiesel, Biogas, etc.
2G biofuel	Non-food crops, waste cooking oil, wood, wood waste, energy crops etc.	Biodiesel, bio-oil, bio-alcohols, etc.
3G biofuel	Microalgae	Biodiesel, vegetable oil, biogas.
4G biofuel	Industrial waste CO_2, captured and recycling carbon, H_20, etc.	Renewable liquid methanol, renewable fossil fuel, etc.

2. Importance of plant biomass

There is a great diversity of plant biomass that can be used as feedstock for production of bioenergy and biofuels, such as forest waste, wood, agriculture and plant species. As mentioned earlier, plant biomass has many possibilities of transforming itself into bioenergy and biofuels, being converted into solid fuel or liquid biofuel (Ioelovich *et al*.,2013). However, it's necessary to consider which crop to grow, which of them have better yield, where it's the ideal place to grow them, how they should be processed and how crop management should be carried out (Landis *et al*.,2018).

Studies report that low yielding crops require a larger land consumption. In this way, to obtain crops with a good yield it's necessary to carry it out in a sustainable way with minimal energy inputs for crop production including cultivation, planting, nutrient production and application, harvesting and transport (Henry.,2010).

Oil-crops are an example of biomass feedstock that can be used to produce biofuels and bioenergy. Oil-crops are plants that are grown to produce oil, these have great diversity and can be divided into two large groups: edible and non-edible vegetable oils. Edible oils are used as feedstock for the 1G biodiesel, which raises many socio-economic issues. As mentioned earlier, first-generation fuels raise food-versus-fuel debate as well as the creation of ecological imbalances when converting forests into agricultural land.

Non-edible vegetable oils are considered as potential alternatives to diesel fuels, such as colza and others (Zanetti *et al*.,2013). Non-edible vegetable oils originates 2G biodiesel from crops that are well adapted to arid and semiarid conditions, as well as, require low fertilizer, little irrigation and are not indicated for human consumption due to the presence of toxic components in the oils (Hu *et al*.,2008). In the USA, soybeans are the main vegetable oil feedstock being the main source for biodiesel while in Europe the main source is the rapeseed (Demirbas., 2007).

Therefore, vegetable oil feedstock has advantages and disadvantages. The advantages are related to environmental benefits, such as reduction of wastes and biodegradability, liquid nature-portability, renewable and potentially inexhaustible source of energy (Demirbas.,2003) (Vassilev *et al*.,2015). On the contrary, the use of vegetable oils as feedstock present several problems due to the high viscosity, low volatility, as well as, combustion deficiencies that give rise to deposits in the fuel injector of diesel engines. It also occurs reactivity of unsaturated hydrocarbon chains, great growing/ harvesting/ collection/ transportation/ storage and pre-treatment costs, difficulties on regional/seasonal availability and local energy supply and high investment cost (Demirbas,2003) (Vassilev *et al*.,2015).

3. Hydrothermal Carbonization

The Hydrothermal Carbonization (HTC) is a thermochemical conversion process which has the great capacity to convert wet biomass into energy and chemicals without pre-drying. The HTC process was discovered in 1913 by *Bergias*, which mimicked the natural process of coal formation (Die., 1913). In the last decades, this process was re-discovered being referred as subcritical water treatment or hydrothermal treatment, which is based on the artificial conversion of the cellulose in materials similar to coal (Wang *et al.,* 2018).

3.1 Characteristics of the process

The feedstock during the HTC process is converted into lignite-like solid product which is constantly affected by the medium. The biomass most used is wet biomass, since requires no pre-drying prior to HTC. There is a wide variety of feedstock that can be used, such as cellulose, glucose, agricultural residue, food waste, aquaculture residues, algal residues and others (Wang *et al.,* 2018). The biomass used in the HTC process undergoes a structural rearrangement by degradation, which originate different types of products: solid (hydrochar), liquid (bio-oil) and gaseous (CO_2). While hydrochar is the main product of HTC process, there is also the formation of an aqueous phase that have organic and inorganic compounds from the feedstock. These compounds are sugar and lignin derivatives which may have numerous applications if recovered, for example in the production of biochemicals and / or biomaterials. (Zhang *et al*.,2015) The proprieties of these products are very influenced by the feedstock and process that originated them (Cao *et al*.,2013).

This thermochemical process occurs in the subcritical region, at high temperatures (180-350ºC) and can occur between 1- 72 hours (Funke *et a.l*, 2010) (Yan *et al*.,2017).The subcritical conditions cause constant changes in the characteristics of the water, which originates a decrease of the dielectric constant, increasingly weak water´s hydrogen bonds and production of high ionization constants. This enhances the dissociation of water into acidic hydronium ions (H_30^+) and basic hydroxide ions (OH^-) (Marcus,1999) (Savage,1999). In addition, the subcritical water can increase the H^+ concentration as compared to liquid water, what causes an excellent medium for the acid-catalyzed reaction of organic compounds. Consequently, these conditions make the water an excellent solvent making the process medium highly reactive (Ruiz *et al*,.2013).

Figure 3.1 demonstrates a representative graph of the subcritical region where occurs the HTC process.

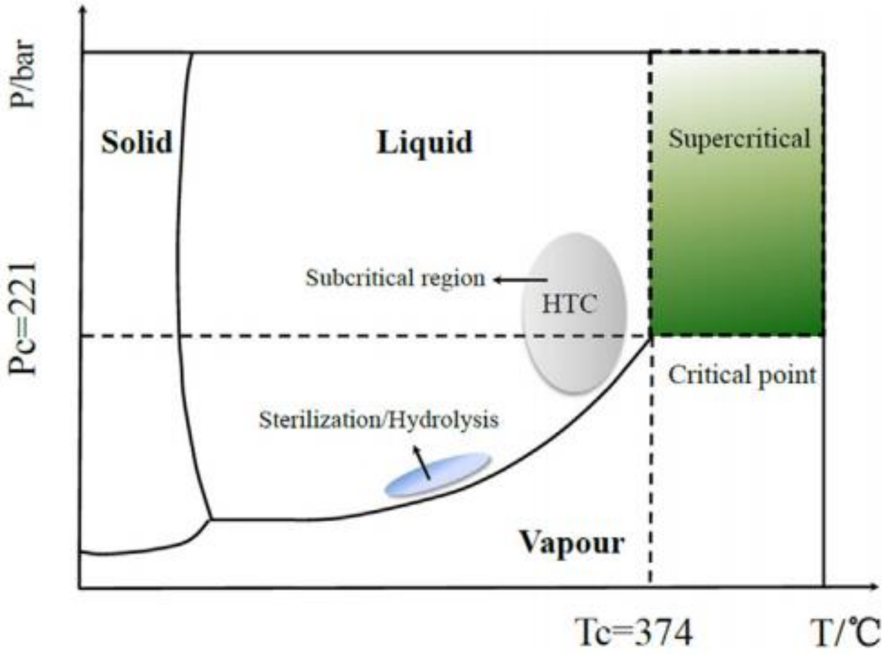

Figure 3.1- Representative graph of the water properties under different temperatures and the application field of hydrothermal process. (Wang *et al.*, 2018)

3.2 Production of hydrochar

As already mentioned before, feedstock undergoes several changes causing different chemical composition and completely different appearance from the original biomass. During the HTC process, there are different chemical reactions that occurs simultaneously and in order such as hydrolysis, dehydration, decarboxylation, aromatization and re-condensation (Funke *et al*.,2010).

The first step of the HTC process is hydrolysis, which breaks down the biomass chemical structure through the cleavage of ester and ether bonds of bio-macromolecules with water molecules. This chemical reaction originates oligosaccharides and fragments of lignin which enter the liquid phase of the HTC process. Then the lignin fragments are hydrolyzed into phenols, however the oligosaccharides are still present in the processing of HTC and can integrate other chemical pathways (Fakkaew *et al*.,2015). The second step in the HTC process is dehydration, this chemical reaction withdraws the water present in the biomass matrix which causes hydroxyl groups to be eliminated. The decarboxylation is the third step which consist of the elimination of carboxyl groups in the HTC process. The fourth step is aromatization this is the consequence of dehydration and decarboxylation. It also occurs the replacing of single bonded hydroxyl and carboxyl groups present in the biomass matrix into double bonded functional groups. These two mechanisms give rise to furfural compounds which then undergoes hydrolysis producing several compounds, such as phenols, aldehydes and acids. These last ones catalyze the release of inorganic elements from the biomass matrix (Fang *et al*.,2018). The compounds mentioned before may undergo re-condensation if reactive. The lignin fragments from the plant biomass are highly reactive and condensate, as well as, aromatized polymers present on the degraded cellulose fragments (Kang *et al*., 2016). However, the degradation of hemicellulose products stabilizes lignin fragments and slow down condensation reactions. In this way, the re-condensation of degraded products from HTC originates hydrochar (Libra *et al*., 2011). Figure 3.2 presents an example of physical transformation of wood sample to hydrochar.

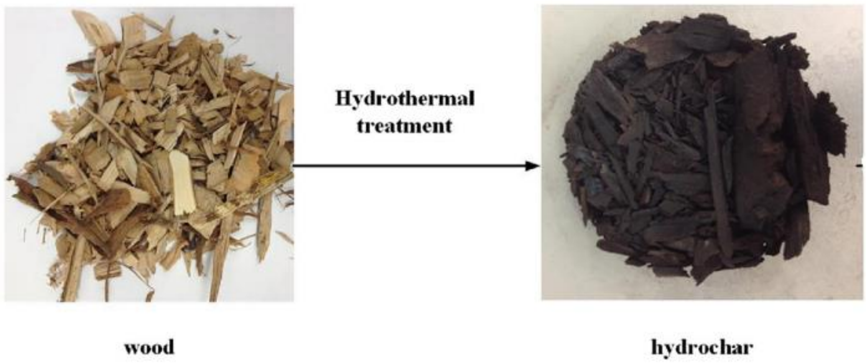

Figure 3.2- Hydrochar produced from a wood sample-*Euc Amplifolie.* (Fang *et al*.,2018)

3.2.1 Properties of hydrochar

The stages of dehydration and decarboxylation during the process cause hydrochar to generally have a higher proportion of carbon (C) and a lower proportion of oxygen (O) compared to the raw biomass, as well as, the formation of aromatic carbon (Funke *et al*., 2010). The inorganic elements are released during biomass degradation and dissolved in the processing liquid during the HTC process causing a lower ash content in hydrochar than biomass (Mäkelä *et al*.,2015). Thus, the low ash content present in the hydrochar, makes it the most suitable precursor for the activated carbon (Fang *et al*.,2018). In addition, sulfur (S) and nitrogen (N) contents are low because of the dissolution of nitrogen and sulfur oxides in the processing liquid during HTC process (He *et al*.,2013).

3.2.2 Applications of the hydrochar

Hydrochar is a product that has been studied in numerous investigations due to its great application potential. Hydrochar can be used as a soil amendment, because it can sequester carbon present in GHGs, such as N_2O, CO_2 and CH_4. In recent studies, it was concluded that hydrochar can increase NH_3, CO_2 and CH_4 emissions from the soil in comparation to unamended soil (Subedi *et al*., 2015) (Malghani *et al*.,2013). The increase of the CO_2 emissions is due to low carbon stability, as well as, the high degradability which causes a high microbial activity stimulation (Kammann *et al*., 2012). Another application of hydrochar is using it as a soil fertilizer to be added to the soil and increase the fertilizer effectiveness. (Bargmann *et al*., 2014).Hydrochar can also be used as a method to retain water, when added to the soil which increases the percentage of water that can be retained by the soil. The reduction of soil density and increase total pore volume, allows a better water retention in the soils although it´s not as effective as the addition of biochar (Abel *et al*., 2013) (Fang *et al*.,2018). In addition to that, hydrochar can also be used as heavy metal removal. In this case, hydrochar can be modified with chemicals improving its sorptive abilities for a great variety of heavy metals. (Sun *et al*., 2015). Finally, hydrochar can be used as a removal of organic pollutants allowing the absorption of organic products such as dyes, pharmaceuticals and pesticides (Zhu *et al.*, 2014) (Mestre *et al*., 2015).

3.2.3 Opportunities and constraints of hydrochar

The constraints related to the use of hydrochar are mainly due to the reduced efficacy compared to the use of biochar. Several studies concluded that biochar has a synergistic effect with fertilizers, thus improving fertilizer efficiency and consequently enhancing plant growth.(Yao *et al*., 2012).In addition, hydrochar and its derivates can display adverse effects such as ecotoxicity and environment impacts that need more detail analyses, as well as, the difficulty to keep it to an ideal humidity for the use in the soils without any fungal degradation. Therefore, it´s necessary additional research in this topic. On the opposite, hydrochar also presents numerous possibilities such as sustainable alternative for the use of high moisture waste biomass, as well as, high potential for being used in various applications as mentioned above (Fang *et al*.,2018).

4. Phytoremediation

Environmental pollution is one of the most demanding problems of the world presently, being one of them the presence of heavy metals in the environment (water, soil and wastewater). There are several conventional physical and chemical remediation methods, such as soil incineration, excavation, soil washing, soil flushing, solidification and stabilization of electro-kinetic systems which have many limitations like high cost, irreversible changes in soil properties, disturbance of native soil microflora, intensive labor, etc. (Ali *et al.*, 2013) (Sheoron *et al.*,2011). A greener alternative approach to this problem is phytoremediation, which consists on the use of plants and associated soil microbes with the aim of reducing the concentrations or toxic effects of contaminants in the environments (Greipsson.,2011). These contaminants can be diverse, such as heavy metals, radionuclides, organic pollutants like pesticides, polynuclear aromatic hydrocarbons and polychlorinated biphenyls. This environment friendly method offers low installation and maintenance cost, application in large areas is practicable, profitable and *in situ* applicable (Van., 2009).

4.1 Mechanisms of phytoremediation

There are many different strategies of phytoremediation: one of them is phytoextraction which consists of the plant's ability to perform uptake of contaminants present in soil or water and their translocation and accumulation in aboveground biomass, such as leaves (Ali *et al.*, 2013). The second strategy is phytofiltration which is based on the plant´s removal capacity of contaminants presents on surface waters or wastewaters. The plant can absorb the contaminants, minimizing their movement to underground waters. (Ali *et al.*, 2013) (Sekara *et al.*,2005) The third strategy is phytostabilization (or phytoimmobilization) it’s based on the use of plants for stabilization of contaminants present in certain contaminated soils. The main objective of this strategy is to reduce the mobility and bioavailability of contaminants in the environment. In this way, this method is preventing them to travel to groundwater or to enter in the food chain (Erakhrumen., 2007) (Singh., 2012).

Phytovolatilization is another strategy that consists in the uptake of contaminants present in the soil by plants. These are subsequently converted to volatile form, being released into the atmosphere. However, this method has some drawbacks because the contaminant isn´t completely removed, it only is transferred from the soil to the atmosphere having the problem of being deposited again (Padmavathiamma *et al.*, 2007). Phytodegradation is based on the degradation of organic contaminants through the enzymatic action of different enzymes of the plant, namely dehalogenase and oxygenase (Vishnoiand *et al.*, 2008). Another strategy that is quite like the previous one is the rizodegradation. This strategy is based on the breakdown of organic contaminants present in the soil by microorganism’s characteristic of the rhizosphere. The rhizosphere stimulates the microbial activity by the secretion of amino acids, flavonoids and carbohydrates which are carbon and nitrogen sources to the soil microorganisms (Mukhopadhyay *et al*., 2010).

Lastly, phytodesalination is a recent strategy that uses halophytic plants with the aim of removing salts from salt-affected soils allowing the normal plant growth. These halophytic plants have better capacity to manage heavy metals than glycophytic plants (Manousaki *et al*., 2011) (Sakai *et al*., 2012). Figure 4.1 shows a diagram of the different phytoremediation mechanisms and where these are performed in the plant.

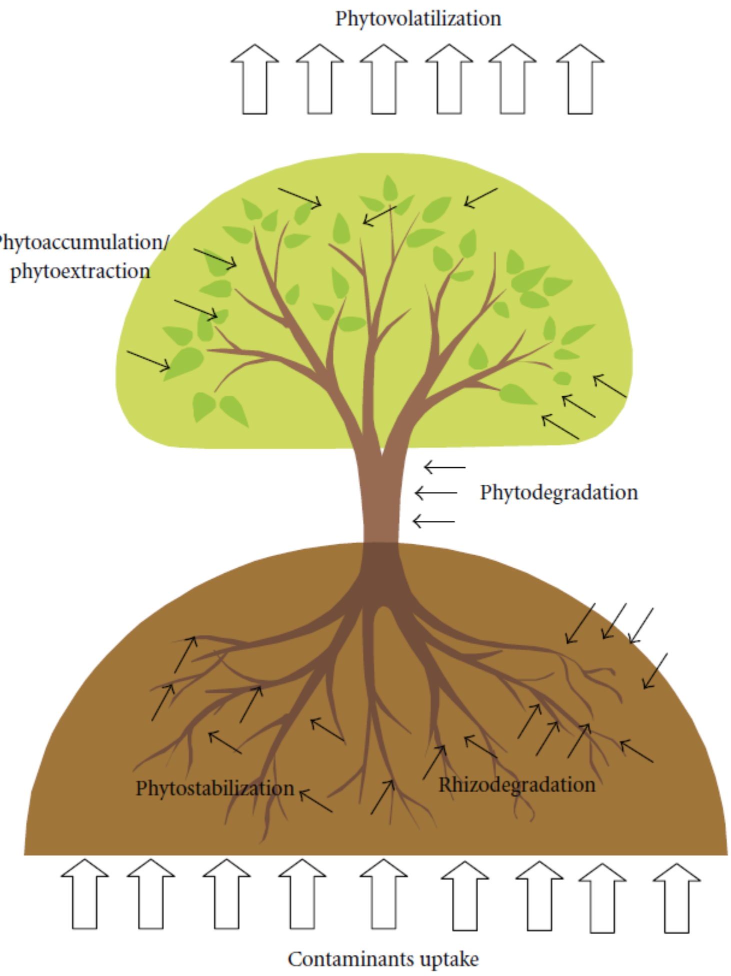

Figure 4.1- Scheme of the different phytoremediation mechanisms performed by the plant. (Tangahu *et al*., 2011)

4.2 Characteristics of phytoremediation plants

One of the main characteristics of the plants that perform phytoremediation is to promote a favorable environment to the diverse microbial population present in the rhizosphere. The microbial population subsists based on the nutrients present in the roots, promoting the degradation of biomass and root exudates. These root exudates stimulate co-metabolic promoting the degradation of organic contaminants present in the soil. Furthermore, the plant´s root have the function of allowing easy and efficient access to contaminants present in the subsurface soil and water. Extensive root system with a large surface area improves the absorption of contaminants present in the soil even if they are at a great depth (Suresh *et al*., 2004). However, there are different factors that affect the uptake of contaminants by the plant, especially the uptake of organic chemicals. The limitations mainly focus on physicochemical properties, such as water solubility, root system, molecular weight, organic and water content in the soil, vapor pressure and octanol/water partition coefficient (Paterson *et al*.,1990). The limitations of phytoremediation are mainly focused on the difficulty of growing plants in environments where there are high toxic levels of contaminants, as well as, when contaminants are present below the depth of rooting (Salt *et al*.,1995).

4.3 *Camelina sativa*

4.3.1 Characteristics

Camelina sativa is part of the Brassicaceae family and is commonly known as “false flax” or “gold of pleasure” (Hutcheon *et al*. 2010). This plant dates back from the Bronze Age in Scandinavia/Western Europe and was grown as an agricultural crop in European countries and in Russia before the Second World War until the mid-twentieth century. This plant has two varieties, spring and winter varieties, that give rise to morphological differences in the shapes and colors of the leaves and siliquae. The differences between the two varieties are mainly due to development of the plant, winter hardness and growth (Zubr, 1997). *Camelina sativa* has a very short life cycle of approximately 120 days from sowing to harvest, and soon after there is the formation of two cotyledon leaves. Immediately after this stage, there is the formation of a rosette of leaves (6-8 leaves) which then gives rise to an erect stalk of many leaves. In the final stage of growth, there is the development of flower buds and axial branches which develop into yellow flowers. These plants can reach 70-100 cm in high. In addition, *C. Sativa* is self-pollinated, originate fruits called siliquae that contain 15-20 seeds which are extremely small (0.7 mm x 1.5 mm). Figure 4.2 presents *C. sativa* in a field and figure 4.3 represents the development of siliquae.

Figure 4.2- *Camelina sativa* in a field. (Source: Russo,2013)

Figure 4.3- Development of siliquae.

4.3.2 Cultivation and growth

C.sativa is a very adaptable plant, since it has the capacity to grow in different climatic and soil conditions, except in heavy clay soils and organic soils. Concerning the cultivation of this plant, it's necessary to prepare a seedbed with good conditions to have a uniform germination. In this way, the depth of the planting shouldn´t be more than 2-3 cm with a row spacing of 13-15 cm. Furthermore, it's necessary to remove all the weeds that can grow because they will compete with the crop. In conclusion, *Camelina sativa* has the possibility to grow as an annual summer crop or as biannual winter crop (Zubr.,1997).

4.3.3 *Camelina sativa* oil

To obtain the oil present in the siliquae of *Camelina sativa*, it's necessary to initiate the processing on an industrial scale. The first steps of the process are crushing and pressing of the seeds, this last step occurs at very high temperature (100ºC). From the pressing step, the oil (crude oil) obtained has a yellowish tint and a characteristic taste, being necessary to be refined to be used in several applications. After that, the crude oil is refined trough filtration, which is a pre-treatment for the step of deodorization, as well as, avoids by-products such as polluted water, polluted bleaching soil and others (Zubr.,1997). Deodorization is very important step, because removes many oxidation and odoriferous compounds that exhibit organoleptic properties that aren´t desirable in dietary or in cosmetic applications (Hrastar *et al*., 2011). This major step consists of a steam distillation process carried out under vacuum and at high temperatures ranging from 210-270 ºC (Erickson.,2007).

The *Camelina sativa* oil has a very high percentage of unsaturated fatty acids (90%). The oil has approximately 15% of linoleic acid (C18:2), 15% of gondoic acid (C20:1) and 3% of erucic acid (C22:1). There is also α-linolenic acid (C18:3) corresponding approximately 35% of the total fatty acids (Zubr.,1997).

4.3.4 Applications of *Camelina sativa* oil

Camelina sativa oil has many applications, many of which have great economic potential and embrace completely different areas. One of them is in human nutrition. This area focuses mainly on the great problem that is the nutritional deficiency currently in human food. The main cause of this problem is the deficiency of poly-unsaturated in the diet of the population of industrialized countries. However, it's possible to reverse this problem by adding into the human diet oils rich in n-3 fatty acids, such as *C. sativa* oil. As discussed above, *C. sativa* oil is richer in polyunsaturated fatty acids and n-3 fatty acids compared to other vegetable oils, such as sunflower oil or olive oil, therefore advisable to introduce it into various recipes (Warich *et al*.,2013) (Zubr.,1997). A very surprising area that *C. sativa* oil can be used is in medicine. This oil has beneficial health effects such as improving the nerve tissue and retina. That is, the existence of antioxidants and alpha linoleic acid (ALA) in the oil brings benefits to the human health. This last component can improve the n-6/n-3 fatty acid ratio and it's an existing component of the human metabolism (Warich *et al*.,2013). Also, this oil can improve the regeneration of skin elasticity and of cells making this oil suitable for skin creams and lotions (Zubr.,1997) (Vollmann *et al*.,1996). Finally, *C. sativa* oil can be used as feedstock in the production of biodiesel. In recent years, studies have shown that *C. sativa* oil can substitute rapeseed´s oil. That is, *C. sativa* oil has the same properties as rapeseed´s oil, but it has the great advantage of having a high iodine content that protects against the faster deterioration of the lubricating oil (Warich *et al*.,2013) (Frohlich *et al*.,2005). However, fuel tests in Europe and refereed by Bernardo *et al* (2003) mentioned that *C. sativa* oil has higher fuel consumption and high O_2 and NO emissions. Nevertheless, it has been shown by several studies that *C. sativa* is a safe and environmentally beneficial choice as a biodiesel crop due to its reduced life cycle as well as low emissions compared to diesel fuel (Krohn *et al*.,2012).

4.3.5 Phytoremediation

The family Brassicaceae has several plants that have phytoremediation properties, such as *Arabidopsis thaliana* and *Thlaspi caerulescens*. Studies carried out by Grispen *et al*. (2006) proved that these plants accumulate a high concentration of Zn and Cd, so these plants have been studied in several investigations on phytoremediation.

Thus, as *Camelina sativa* belongs to the same family of plants, it has been recently studied to verify if it has phytoremediation capacity. One of these studies was carried out by Putnik-Delić *et al*. (2013) with the main objective of finding out if *Camelina sativa* can phytoremediate heavy metals. The research group concluded that in fact *C. sativa* is capable of phytoremediate heavy metals present in the medium, since the plant showed an increase in the concentration of these metals.

Another study carried out by Popa *et al.* (2018), aimed to determine if the residual pesticides present in the soil were concentrated in the siliquae of *Camelina sativa*. They concluded that the pesticides aren´t concentrated in the siliquae, allowing the safe use of *C. sativa* siliquae for various purposes even when grown in contaminated soils. In addition, the study concluded that the cultivation of *Camelina sativa* in contaminated soils (DDT and its metabolites) minimizes the risk of pesticides translocation.

5. Aim of the work

The main objective of this work is to evaluate the phytodepuration capacity of *Camelina sativa* (winter and spring varieties) subjected to irrigation with contaminated effluents from the HTC process. In addition, to evaluate if it's possible to reuse these effluents in the production of *Camelina sativa.*

As previously mentioned, during the hydrothermal carbonization process (HTC) toxic inorganic and organic compounds are leached into a liquid phase originating contaminated effluents. To depurate these effluents in a sustainable and environmentally safe way, phytoremediation is a good alternative.

Phytoremediation is the use of plants *in situ* to remove or remedy the pollutants present in the contaminated effluents. In addition, the use of *Camelina sativa* will allow to evaluate the effect of irrigation with contaminated effluent from the HTC process on the production and biomass quality of the plant. As well as, to evaluate if there are changes in the quality of the oil produced by *Camelina sativa*, since this plant is widely used for energy purposes at the level of bioeconomic and circular economy.

The phytodepuration capacity of *Camelina sativa* will be evaluated following experimental planning in pots where two varieties of *Camelina sativa* will be sown: winter and spring varieties.

In this way, the experimental have the following objectives:

- Characterize the contaminated effluents and assess possible risks to watering plants;
- Analyze the effect on yield and biomass quality of *Camelina sativa*;
- Evaluate the phytodepuration capacity of *C. sativa* in the removal of the contaminants present in the effluents;
- Characterize the percolated waters collected from the soil system pots and from the soil-biomass system pots, and to evaluate the possible risks of contamination in the groundwater;

6. Experimental Methodologies

The present methodologies have as main objective to evaluate the phytodepuration capacity of *Camelina sativa* which is subject to the irrigation of contaminated effluent from the aqueous phase of HTC process. Thus, the following experimental analysis aim to achieve the following objectives:

- To analyze the effect of the HTC effluent in the growth, productivity and quality of *Camelina sativa* biomass;
- To determine the efficiency of phytodepuration, that is the capacity to depurate the contaminates present in the HTC effluent;
- To study the reutilization of HTC effluents on the production of *Camelina sativa*;

6.1 Physical-chemical characterization of HTC effluent

The water used in this work comes from HTC effluent, which is a mixture of process water and coal wash water. The hydrothermal carbonization (HTC) process was carried out in the FCT/NOVA facilities at high temperatures (250º-350ºC) and lasted approximately 30-120 minutes.

The biomass used for this process is a mixture of lignocellulosic RDF (Refuse Derived Fuels) consisting of pellets, polymeric RDF (mainly industrial waste), pine and eucalyptus biomass. To achieve the objectives mentioned above, it’s necessary to carry out a careful study of the various components present in the effluent from the HTC process. The different methods used in the characterization of the effluent are described in the table 6.1 and table 6.2.

Table 6.1- Analytical methods used in the characterization of the samples from the HTC effluent

Parameters	Analytical methodology
pH	Electrometry. (APHA *et al*., 1985) The pH apparatus was previously calibrated with two buffer solutions of pH 4.0 and 7.0.
Conductivity	Determination through a conductivity meter. (ISO 7888:1985)
Total alkalinity	Alkalinity is determined by end point titration with a strong acid solution. (ISO 9963-1:1994)
Total acidity	Determination of total acidity by titration with 0.02 N NaOH.
Nitrite, Nitrate	Reduction of nitrates to nitrites by contact of the filtered extract with zinc powder. Determination of nitrites by molecular absorption spectrophotometry through the formation of a red-purple complex by the combination of diazotized sulfanilic acid with NED. (ISO 6777: 1984; Jenkins *et al*., 1996)
Ammonium ion	Distillation and subsequent titration of the distillate with 0.02 N HCl. (ISO 5664:1984)
Total nitrogen	Kjeldahl method: Mineralization with H_2SO_4, distillation and titration of the distillate with H_2SO_4 0.02 N (Watts *et al*., 1996).
Oil and fat	Extraction (three times) and gravimetry with hexane in a separatory funnel. The extract is dried with sodium sulfate, the solvent and any volatile components are evaporated, and the residue is weighed. (United States Environmental Protection Agency (EPA).,1999)
BOD_5	Determination of the consumption of dissolved oxygen after 5 days of incubation at 20ºC away from the light with the addition of a nitrification inhibitor. (ISO 5815-1; 2 :2003)
Oxidability	Oxidation by potassium permanganate, at high temperatures, in acid medium, of the organic matter present in the samples. Determination of the permanganate consumed by the addition of an excess of oxalate, followed by titration with permanganate. (ISO 8467:1993)
COD	Oxidation by potassium dichromate in a digestion tube in the presence of mercury sulphate and concentrated sulfuric acid at 160 °C for 110 minutes in a digester. After digestion, excess potassium dichromate is titrated with ferrous sulphate using ferroin as indicator. (APHA *et al*.,1985)
Total solids	Determination of total solids by evaporation in a water bath followed by drying at 105ºC for 1 hour. Weighing before (capsule) and after the process (capsule plus sample). (APHA *et al*., 1985)
Total fixed solids	Samples are calcinated at 550ºC for 1 hour. Determination of total fixed solids by the difference between the weight after calcination (capsule plus ash) and the weight of the capsule. (APHA *et al*., 1985)
Total volatile solids	Determination of total volatile solids by the difference between the total solids and the fixed solids. (APHA *et al*., 1985)

Table 6.2- Analytical methods used in the characterization of the samples from the HTC effluent (continuation)

Parameters	Analytical methodology
Total suspended solids	Determination of total suspended solids by filtration through 0.45 µm filter membrane, drying at 105 ° C and weighing. (APHA *et al*., 1985).
Color	Determination of the color of the sample by a photometer. Adaptation of APHA *et al*., (1992).
Total Phosphorus Orthophosphates	Total Phosphorus: Hot digestion with H_2SO_4. Determination of phosphates in the digested. Phosphates: Formation of phosphomolybdate complex. This complex is reduced by ascorbic acid in the presence of Antimony potassium tartarte originating a colored molybdenum blue complex. Determination of orthophosphates and phosphorous by spectrophotometry. (Watanabe et al., 1965; ISO 6878:2004) (Watts et al., 1996)
Total phenolics	Determination of total phenolics by molecular absorption spectrophotometry. Adapted from the methodology proposed by Singleton *et al* (1999).
Zn; Cu; Mn; Fe; Ca; Na; Mg; K; Ni;Cd;Pb;Cr	Mineralization of the samples by dry process (incineration in muffle at 550 ± 50ºC,2h) and dissolution of the ashes with HNO_3 (Vandecasteele *et al*., 1993). Determination of metals in extracts by atomic absorption spectrophotometry. Ca and Mg according to ISO 7980 (1986). Na and K according to ISO 9964 (1993). Cu and Zn by ISO 8288 (1986), when the flame was used. Fe and Mn with flame, and the renaming metals according to APHA *et al*. (1985).

The HTC effluent was collected and stored in plastic containers in mid-June of 2018, according to the norm ISO 5667-3:2018 until all analysis were complete. The analysis was performed in duplicated. The analytic methods described in table 6.1, table 6.2 and analysis by GC / MS have as main objective to analyze which are the organic compounds present in the effluent of HTC, as well as, later find out if *Camelina sativa* has phytodepuration capacity.

6.2 Preparation of the pot essay and physical -chemical characterization of wastewaters and percolated waters

Chemical oxygen demand (COD) was determined in the HTC effluent to define the irrigation levels. Considering the COD value obtained for the raw effluent (360 mg O_2/L) it was decided to dilute it in order to obtain the limit value of discharge established by the Decree Law 236/98 (Portuguese regulation that establishes norms, criteria and objectives of water quality according to its main uses): 150 mgO_2/L. The limit COD value was set as WW2. WW1 corresponds to 80% of the limit value (120 mg O_2/L) and WW3 corresponds to an increase of 20% of the limit value (180 mg O_2/L). In a parallel essay the crops were irrigated with tap water as control. Five replicates were set for each trial (WW1, WW2 and WW3) and also for control (48 pots in total, 8 without plants)

In mid-November of 2018, *Camelina sativa* seeds of both varieties (spring and winter) were sown in pots. For each pot, was added about 1.50 kg of soil from the vicinity of the FCT/UNL, as well as, added 20 Camelina sativa seeds. The pots (with and without plants) were fertilized with different compounds: 3 g N/m2 (urea, 46% N); 3 g N/m2 (nitrolusal, mixture of NH4NO3 + CaCO3, 27% N); 17 g K_2SO_4/m2 (potassium sulphate, 51% K_2SO_4); 26 g P_2O_5/m2 (superphosphate, 18% P_2O_5).

Thus, in January 2019 the irrigation of the 48 pots was started considering the scheme represented in the figure 6.1.

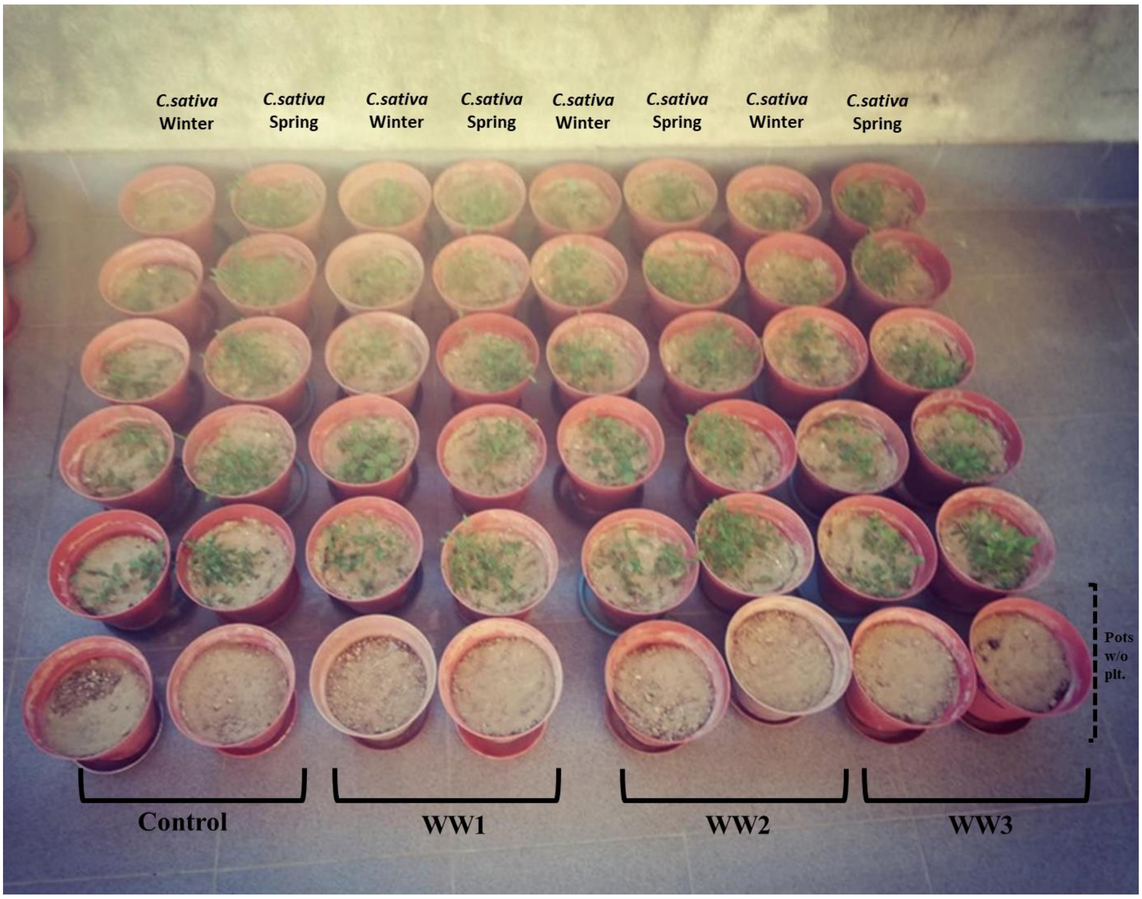

Figure 6.1- Scheme of the irrigation of the pots with the different wastewaters (control,WW1, WW2 and WW3). The dashed line in the figure represents the vases without plants.

Irrigation was applied every week at a rate to avoid water stress. Each pot with *Camelina sativa* (spring and winter varieties) were irrigated with about 50 ml of wastewater, every 2-3 days until the end of February 2019 and with 100 ml, every 2-3 days, until the end of May. At the same time, pots with only soil (control, WW1, WW2 and WW3) were also analyzed to study and to compare the soil-biomass system and the soil system. The pots without plants received also the same amount of water and wastewater in the irrigation as the pots with biomass.

Percolated waters were evaluated along the growing season to evaluate if the soil-biomass system was able to retain the contaminants. From the five pots of each type of irrigation, all the percolated waters were collected and mixed. The characterization of the percolated waters was performed in duplicate for each group of wastewaters (control, WW1, WW2, and WW3). Testing the different wastewaters is of importance, because it allows to study the different responses of *Camelina sativa* to the HTC effluents, with different grades of chemical oxygen demand.

Regarding the analysis of the percolated waters, they were collected monthly during this experiment in the period from January to May of 2019. All the collected percolated waters were filtered and stored, according to ISO 5667-3: 2018, in 250 ml plastic bottles until they were analyzed. The methods used for the analysis of the percolated waters are described in table 6.3, and results obtained will allow to know if *Camelina sativa* has phytodepuration capacity and how the wastewaters can affect this plant.

Table 6.3- Analytical methods used in the physicochemical characterization of percolated waters

Parameters	Analytical methodology
pH	Electrometry. (APHA *et al*., 1985) The pH apparatus was previously calibrated with two buffer solutions of pH 4.0 and 7.0.
Conductivity	Determination through a conductivity meter. (ISO 7888:1985)
Total acidity	Determination of total acidity by titration with 0.02 N NaOH.
Total phenolics	Determination of total phenolics by molecular absorption spectrophotometry. Adapted from the methodology proposed by Singleton *et al* (1999).
Oxidability	Oxidation by reaction with potassium permanganate at elevated acidic temperatures of the organic matter present in the samples. Determination of the permanganate consumed by the addition of excess oxalate followed by titration with permanganate to obtain the result. (ISO 8467, 1993)
Color	Determination of the color of the sample by a photometer. Adaptation of APHA *et al*., (1992).

6.3 Analysis of HTC effluent and percolated waters by GC/MS

First of all, a dispersive liquid-liquid microextraction (DLLME) was performed on the percolated waters (January and May samples) and on the HTC effluent sample (Rezaee *et al.*,2006) (Rezaee *et al.*,2010). Each sample (10 ml) was transferred to a conical bottom centrifuge tube (12 ml) to which about 10 drops of sulfuric acid (10%) was added. To each tube was also added 1 ml of acetone and then homogenized.

To extract the samples, 500 µl of chloroform (99,99 %, Fisher Scientific) was added and then vortexed until a cloudy solution was formed. Afterwards, the samples were centrifuged at 2000 rpm for 1 minute (Hettich EBA 20). After extraction, the extracts were dried with anhydrous sodium sulfate (≥ 99%, LabKem), evaporated to dryness at room temperature, derivatized with 5 µl of bis (trimethylsilyl) acetamide (BTMSA, Sigma) and re-dissolved in 50 µL of chloroform. The samples were stored in 2 ml *vials*, at -5 ºC until analysis.

The extracts were analyzed by GC-MS to identify the organic compounds present in the percolated waters and on the HTC effluent. Thus, about 2 µl of the sample were injected for analysis by GC-MS. Separation was performed on a DB-5 column (30 m long, 0.25 mm wide and 0.25 µm film thickness). The carrier gas flow (helium 99.999%, Air Liquid) was 1 ml / min, the interface was thermostated at 250 ° C and the ion source was maintained at 240 °C. Acquisition was done in full scan mode and identification of organic compounds was per-formed by comparison of retention times and mass spectra with analytical standards and reference mass spectra libraries (NIST, Wiley).

6.4 Physical-chemical characterization of biomass

The biomass was harvested in the end of May 2019 and the characterization of *Camelina sativa* is divided in morphological parameters and physical-chemical parameters. The morphological parameters are listed in table 6.4 and the physical-chemical parameters are listed in table 6.5. All the different fractions of the plant were characterized physico-chemically.

Table 6.4- Methodology used for morphological parameters

	Camelina sativa Structures				
Parameters	**Leaves**	**Stalk**	**Roots**	**Siliquae**	**Methodology**
Length		x			Measurement with tape measure
Number				x	Counting
Weight	x	x	x	x	Determination by weighting

In addition to the information presented in the table 6.3, the data collected allows to calculate each fraction productivity, calculated from equation 1 expressed in g/m^2. The dry weight is measured from the initial weighing (wet weight) after drying the biomass in a vacuum oven at 40 ° C for 48 hours.

$$Productivity\ (g.m^{-2}) = \frac{dry\ weight\ of\ plant\acute{}s\ component\ (g)}{pot\ area\ (m^2)} \quad (\boldsymbol{Eq.1})$$

Table 6.5- Methodology used for physical-chemical characterization of biomass

Parameters	***Camelina sativa* fractions**				**Methodology**
	Leaves	**Stalk**	**Roots**	**Sili-quae**	
Humidity	**x**	**x**	**x**	**x**	Weight loss after drying at 105 ± 2ºC (2h), repeated until constant weight. (AOAC., 1990).
Ashes	**x**	**x**	**x**	**x**	Mineral residue obtained after incineration at 550 ± 50ºC (2h) (AOAC., 1990).
Total nitrogen	**x**	**x**	**x**	**x**	Kjeldahl method: Mineralization with H_2SO_4, distillation and titration of the distillate with 0.02N HCL (Watts *et al*., 1996).
Total phosphorus	**x**	**x**	**x**	**x**	Hot digestion with HNO_3 and H_2SO_4 (Watts *et al*., 1996). Determination of the phosphates in the digested by molecular absorption spectrophotometry by forming a complex stained with a solution of ammonium molybdate in the presence of ascorbic acid and potassium and antiammonium tartrate (Watanable *et al*., 1965).
Zn; Cu; Mn; Fe; Ca; Na; Mg; K	**x**	**x**	**x**	**x**	Mineralization of the samples by dry process (incineration in muffle at 550 ± 50ºC,2h) and dissolution of the ashes with H2NO3 (Vandecasteele *et al*, 1993). Determination of metals in extracts by atomic absorption spectrophotometry. Ca and Mg according to ISO 7980 (1986). Na and K according to ISO 9964 (1993). Cu and Zn by ISO 8288 (1986), when the flame was used. Fe and Mn with flame, according to APHA *et al*. (1985).

6.5 Equipment and statistical analysis of results

The equipment used in the different analysis described in points *6.1,6.2,6.3 and 6.4* were:

- Determination of conductivity was obtained from the apparatus *METTLER TOLEDO – InLab 730 Conductivity*;
- Determination of nitrite and nitrate was obtained from the molecular absorption spectrophotometer *SPEKOL® Spectrophotometer UV 1500* (540 nm);
- Determination of ammonium ion and total nitrogen was obtained from *DK 6 Heating Digestor VELP SCIENTIFICA* and *Kjeltec System 1002 Distiling Unit Tecator;*
- Determination of total phosphorus and orthophosphates was obtained from the molecular absorption spectrophotometer *SPEKOL® Spectrophotometer UV 1500* (880 nm);
- Determination of total metals was obtained from atomic absorption spectrophotometer *Pharmacia NOVASPEC® II*;
- Determination of total phenolics was obtained from the molecular absorption spectrophotometer *SPEKOL® Spectrophotometer UV 1500* (760 nm);
- Determination of the color of HTC sample and percolated waters was obtained from the multiparameter photometer with COD *HANNA (HI83399)* (420 nm);
- Centrifugation of percolated waters and HTC effluent samples using the centrifuge *Hettich EBA 20*;
- Determination of organic compounds present in HTC effluent and in percolated waters through gas chromatography *Focus GC, PolarisQ, Thermo.*

The mathematic models for the statistical treatment were obtained from:

- ANOVA allows to evaluate the analysis of variance, depending on the number of factors and the number of population samples to be studied;
- Test t allows to determine if it's likely that the two samples with unequal variances under study will result from distributions with equal population averages.

7. Results and Discussion

7.1 Characterization of HTC effluent

The characterization of the HTC effluent, carried out in September 2018, was based on the physical chemical analysis presented in table 6.1 and table 6.2.The results of these experimental analysis were compared with the emission limit values (ELV) established by the annex XVIII from the decree- law no.236/98 concerning wastewater discharges. In this way, the results obtained, and the values established in the Annex XVIII are shown in the table 7.1 and table 7.2.

Table 7.1-Average physicochemical characterization of HTC effluent and its comparison with results of tap water used in the irrigation of control pots and with Annex XVIII of Decree-Law no. 236/98

Parameters	Expression of results	Tap water	Effluent from HTC process	ELV (Annex XVIII)
pH	Scale of Sorensen	7.5 ± 0.5	7.2 ± 0.2	6.0-9.0
Conductivity	µS/cm	350 ± 50	640 ± 202	-
Oil and Fat	mg/L	-	51 ± 10	15
COD	mg/L O_2	1.5 ± 0.3 (oxidability)	360 ± 52	150
Total Solids	mg/L	-	3825 ± 35	-
Total volatile solids	mg/L	-	1070 ± 10	-
Total suspended solids	mg/L	-	140 ± 60	60
Total fixed solids	mg/L	-	2755 ± 25	-
Phosphorus	mg/L P	-	3.2 ± 0.4	10
Ortophosphates	mg/L PO_4	-	0.08 ± 0.03	-
Nitrite	mg/L (NO_2)	0.027 ± 0.008	9.0 ± 1.0	-
Nitrate	mg/L (NO_3)	42 ± 14	0.6± 0.3	50
Total acidity	mg/L $CaCO_3$	-	107 ± 34	-
Total alkalinity	mg/L $CaCO_3$	-	75 ± 7	-
Ammonium ion	mgNH_4/L	0.18 ± 0.02	0	10
Total nitrogen	mgN/L	-	0	15
BOD_5	mgO_2/L	-	55 ± 5	40

Table 7.2- Average physicochemical characterization of HTC effluent and its comparison with results of tap water used in the irrigation of control pots and with Annex XVIII of Decree-Law no. 236/98 (continuation)

Parameters	Expression of results	Tap water	Effluent from HTC process	ELV (Annex XVIII)
Total phenolics	mg/L C_6H_5OH	-	34 ± 10	0.5
Color	-	-	3190	Not visible at 1:20 dilution
Zn	mg/L	1.0 ± 0.4	1.56 ± 0.04	-
Cu	mg/L	0.09 ± 0.04	0.20 ± 0.01	1
K	mg/L	11 ± 2	11.7 ± 0.5	-
Mn	mg/L	0.018 ± 0.004	0.051 ± 0.007	2
Mg	mg/L	8.4 ± 0.4	13.3 ± 0.4	-
Fe	mg/L	0.006 ± 0.002	0.66 ± 0.16	2
Ca	mg/L	30 ± 8	48.8 ± 0.3	-
Na	mg/L	57 ± 9	204 ± 18	-

From table 7.1 and table 7.2 it’s possible to observe that there are several parameters that present values above ELV established by the Annex XVIII of Decree-Law no.236/98. These parameters are total phenolics, oil and fat, COD, BOD_5 and total suspended solids. Therefore, HTC effluent shouldn´t be discharged without a prior treatment.

The high COD values are probably due to the type of biomass used in the HTC process, which contained pellets, pine and eucalyptus biomass. Likewise, the presence of high values of oil and fat in the HTC effluent is due to the type of biomass used, namely polymeric RFU. On the other hand, the presence of phenols in the HTC effluent is due to the decomposition of biomass during the process, in which phenols are leached into the aqueous phase.

Regarding the water quality control for irrigation established by the Annex XVI of Decree-Law no.236/98, it can be observed that the total suspended solids values (140 ± 60 mg/L) of the HTC effluent exceeds the maximum recommended value (MRV) (60 mg/L). This high concentration of total suspended solids may cause soil clogging and siltation in irrigation networks. However, as this parameter doesn´t have an emission limit values (ELV), it’s possible to use the HTC effluent as irrigation water.

Finally, the COD value of the HTC effluent (360 ± 52 mg/L O_2) is much higher than the emission limit value (150 mg/L O_2) set out in Annex XVIII of Decree-Law no.236/98. Considering the main objective of this work, which is to evaluate the phytodepuration capacity of *C. sativa* in the removal of the contaminants present in the effluent, it was necessary to define the dilutions to be made to the HTC effluent for watering the plants. In this way, the HTC effluent was diluted 1:3 (WW1: 120 mg / L O_2), diluted 1:2,4 (WW2: 150 mg / L O_2) and diluted 1: 2 (WW3: 180 mg / LO_2). The dilutions WW1 and WW3 were defined considering the limit value of WW2 for discharge, which WW1 corresponds to 80% of the limit value and WW3 corresponds to an increase of 20% of the limit value for discharge according to the Annex XVIII of the Decree Law 236 / 98.

In terms of metals content, generally the effluent of HTC presents a higher content of metals than the tap water, and this may happen due to HTC process that somehow dissolves and transfers to this effluent the metals present in the feedstock used in the HTC process. However, metals, such as Ni, Pb, Cd and Cr were not detected in this effluent and also the metals Cu, Mn ad Fe, which are present in the effluent, didn’t show a value higher than the limits imposed for discharge. Moreover, the results of Zn, Cu, Mn and Fe are also lower than the limits imposed for irrigation established by the Annex XVI of Decree-Law no.236/98. Therefore, the presence of metals in the HTC effluent seems to not represent a toxicity problem to the plants or soil.

7.2 Characterization of the percolated waters

Percolated waters collected from January to May 2019 were submitted monthly to physical chemical analysis listed in the table 6.3.

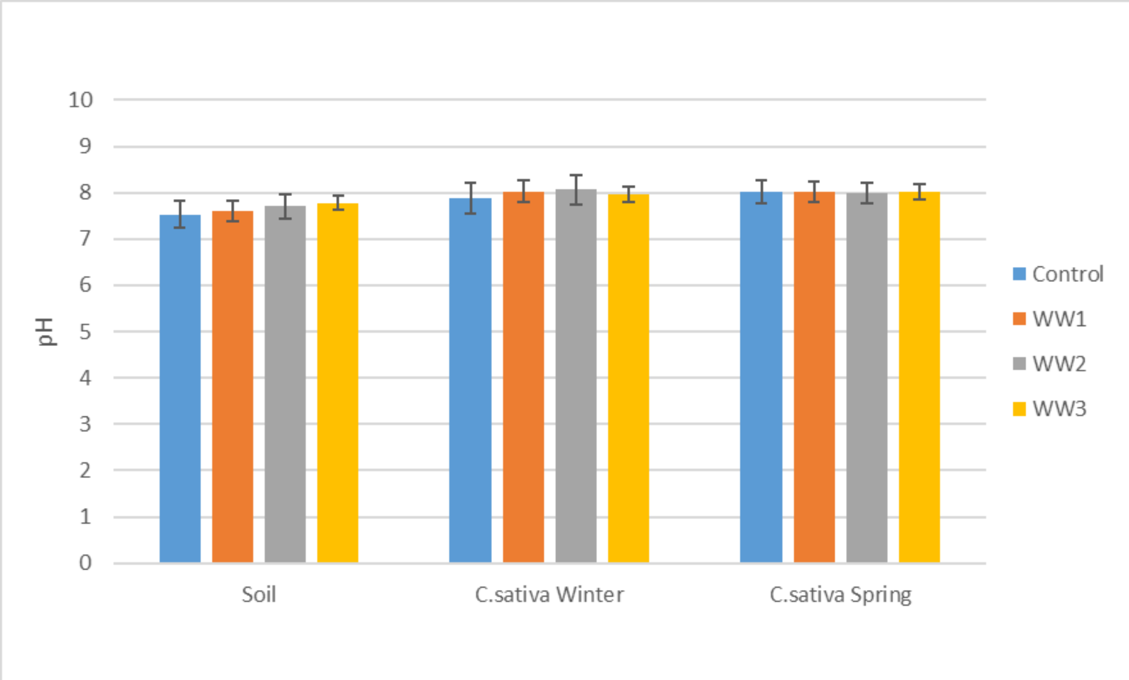

Figure 7.1- Average pH values from the percolated waters of the soil, spring and winter varieties of *C. sativa.*

Figure 7.1 shows the different average pH values from the soil, the spring and winter varieties of *Camelina sativa*. It's possible to observe that the winter variety has the highest pH values, and the highest value corresponds to wastewater WW2 (pH=8.06). While, the spring variety has very uniform values between the different wastewaters (pH=8).

As can be seen, despite the pH values of the percolated waters obtained in pots with soil solely, are lower that the pH values of percolated waters obtained from the pots with both varieties, these aren´t statistically different (p >0.0.5). In general, a slight increase in pH from WW1 to WW3 wastewaters can be observed. This is because WW3 is the least diluted wastewater and therefore has more organic acids from the HTC effluent that increases the pH (Rukshana *et al*.,2010). These organic acids come from the decomposition of lignocellulosic RFU, pine and eucalyptus biomass used in the HTC process.

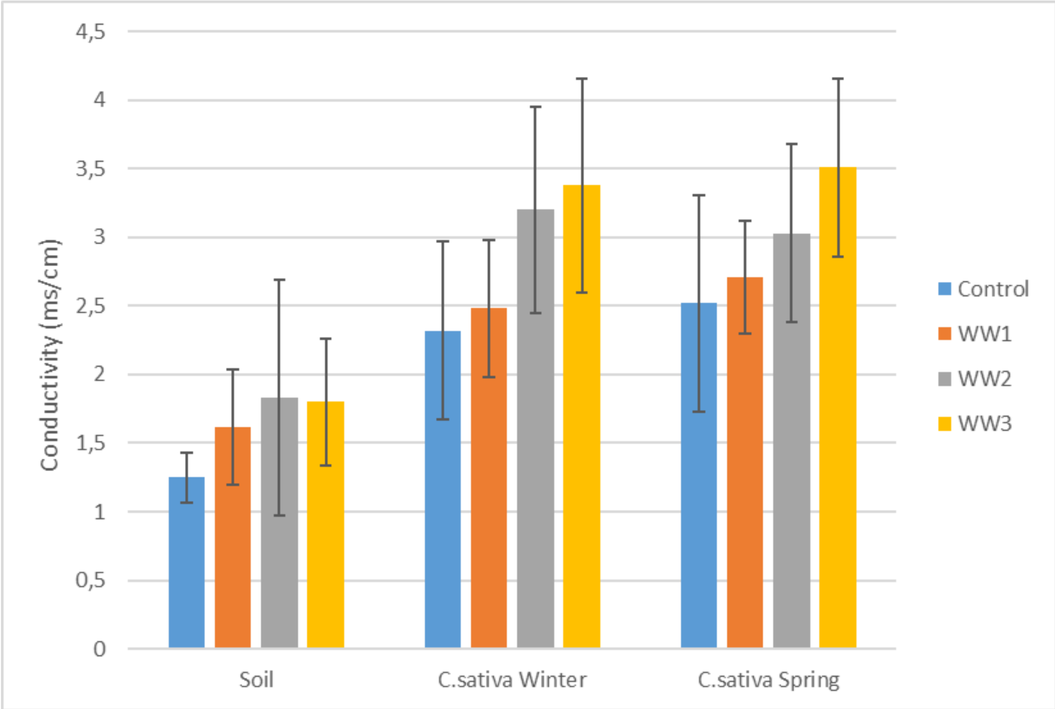

Figure 7.2- Average conductivity (mS/cm) values from the percolated waters of the soil, spring and winter varieties of *C. sativa*.

From the analysis of the figure 7.2 it's possible to verify that both varieties of *Camelina sativa* show similar behavior and conductivity values. In fact, wastewater WW3, in both varieties of *C. sativa*, presents the highest values (3.38; 3.51 mS/cm). It´s possible to observe an increase in conductivity as the load of compounds increases. That is, WW3 has the highest conductivity values as it's the least diluted wastewater, thus presenting a higher load of organic and inorganic compounds. Conversely, the conductivity values of the soil system are much lower.

The results obtained in the soil-biomass system for both varieties, compared with the results of soil solely, may be the result of the combination of several factors. This may be the conjunction of soil retention of Na and K cations from the different wastewaters, as well as, the emission of organic acids by *C.sativa* roots that potentiate the release of ions into the percolated waters (Mojiri.,2011) (Cieslinski *et al*, 1997).

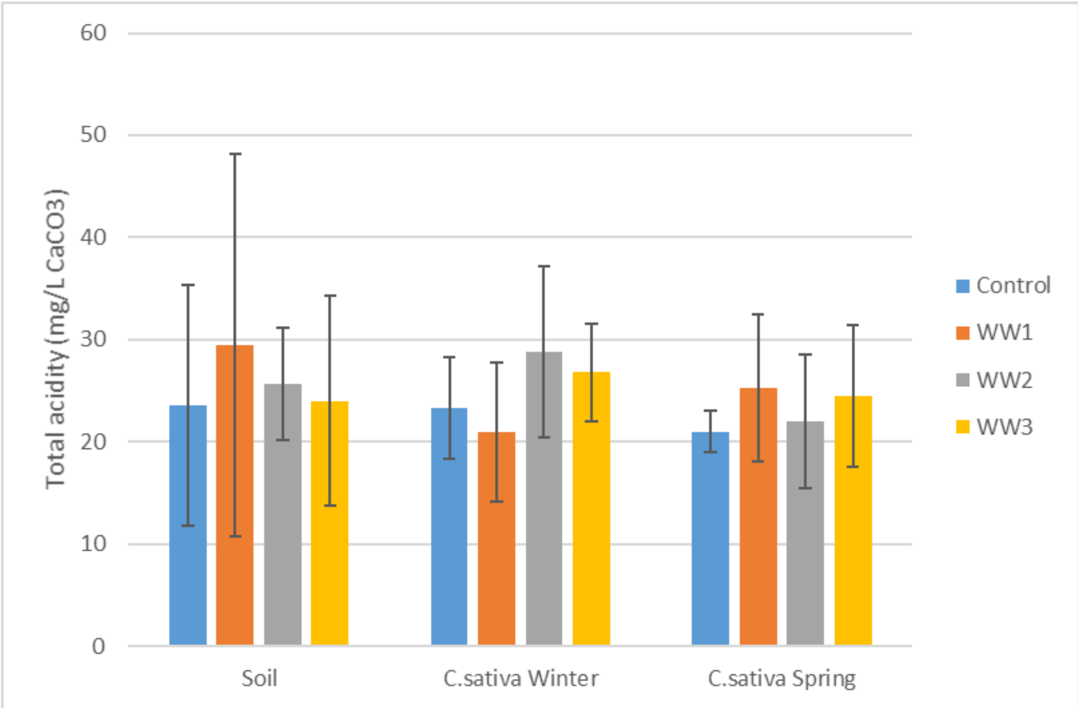

Figure 7.3 Average total acidity values (mg/ L $CaCO_3$) from the percolated waters of the soil, spring and winter varieties of *C. sativa*.

Regarding the analysis of total acidity, it’s possible to verify from figure 7.3 that the soil and both varieties of *C. sativa* had similar behavior. That is, total acidity wasn´t influenced by either *Camelina sativa* (winter and spring varieties) or by the different wastewaters. Nevertheless, there is indeed a decrease in acidity in the percolated waters of the soil-biomass and soil systems compared to the acidity value of HTC effluent (107 ± 34 mg/ L $CaCO_3$).

This may suggest that the soil and soil-biomass systems play the role of pH buffering as there is a reduction in the initial acidity value, as well as, favorable soil conditions, such as air circulation, may have favored the reduction of the initial acidity of the HTC effluent (Irandoust *et al*.,2017).

The acidity detected in the percolated waters from the two systems, may be the remnant of organic acids from the degradation of biomass of the HTC process, namely cellulose and hemicellulose, which weren´t completely depurated (Wilk *et al*., 2017). As well as, the excretion of organic acids from the plant roots, as a source of nutrients for the microbial population present in the root system (Cieslinski *et al*, 1997).

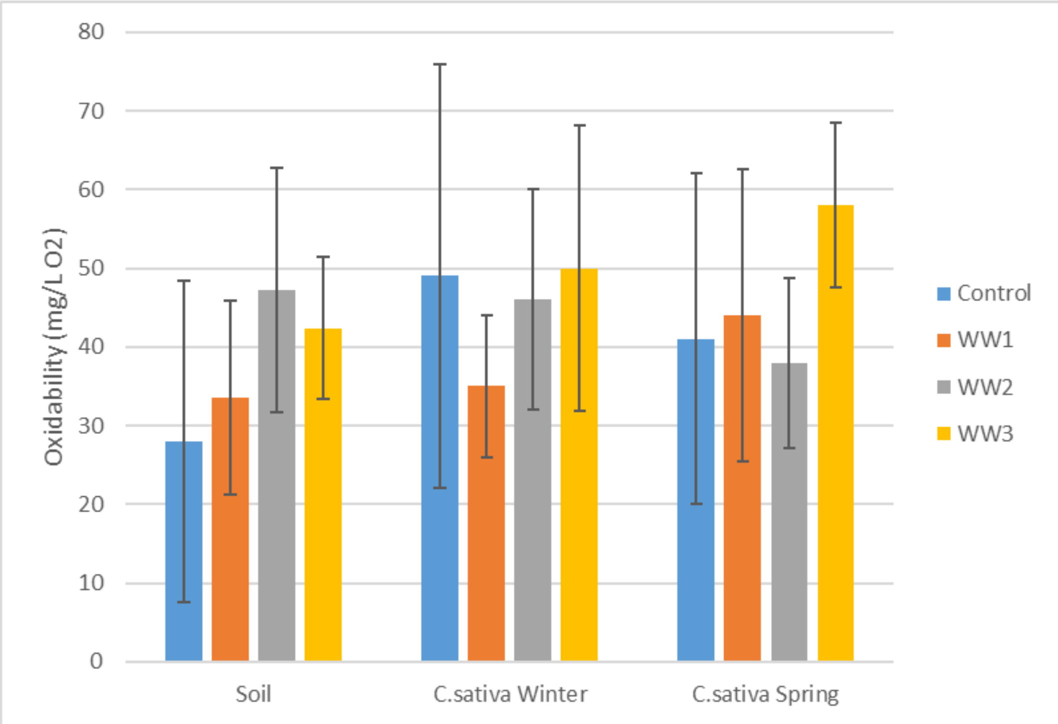

Figure 7.4- Average oxidability (mg/L O_2) values from the percolated waters of the soil, spring and winter varieties of *C. sativa*.

Figure 7.4 shows that both *Camelina sativa* varieties and the soil present lower values than 150 mg/L O_2. Therefore, the percolated waters can be discharge according to the ELV established by the annex XVIII from the decree- law no.236/98.

As can be seen, the soil-biomass system was able to depurate the different wastewaters avoiding the contamination of groundwater. There is a tendency of increase oxidability in the percolated waters as the wastewaters have higher organic and inorganic load, although there aren't statistically significant differences ($p >0.0.5$) between the soil system and the soil-biomass system. Concerning the control, tap water has an oxidability of 1.5 mg / L O_2, and in percolated waters it has much higher oxidability values due to soil leaching of organic and inorganic compounds.

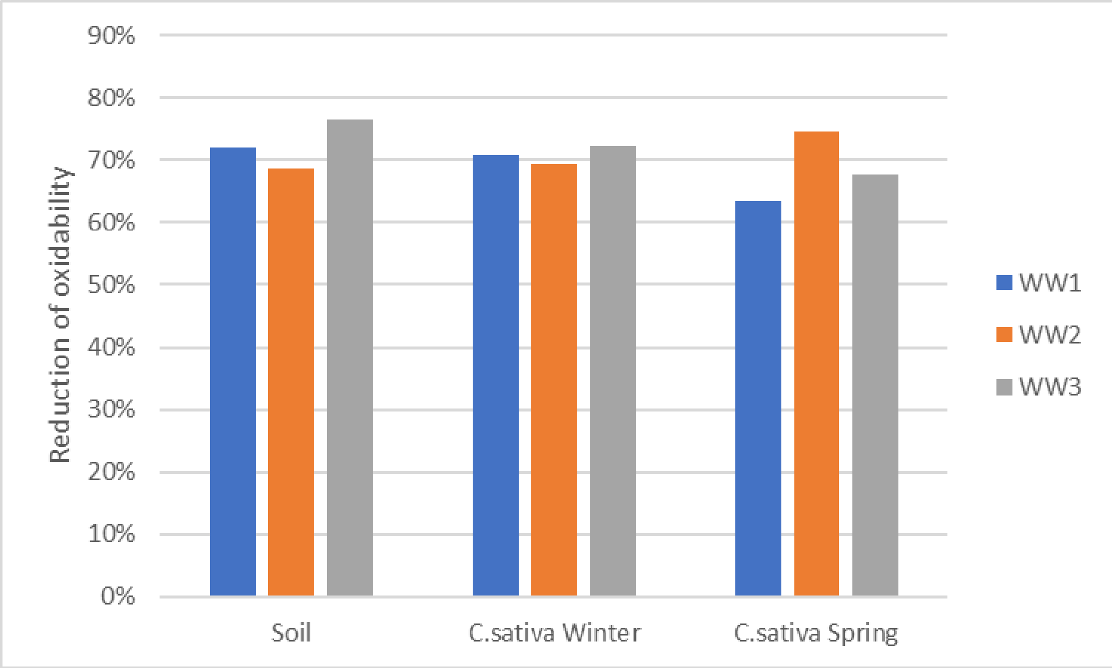

Figure 7.5-- Oxidability decrease (%) from the initial values of the different wastewaters (WW1, WW2 and WW3) obtained from the analysis of percolated waters of the soil and soil-biomass systems.

Figure 7.5 shows the percentages of soil and *Camelina sativa* (summer and winter variants) oxidability decrease when compared to the initial oxidability values of WW1 (120 mg/L O_2), WW2 (150 mg/L O_2) and WW3 (180 mg/L O_2) . From the analysis of the figure 7.5, it's possible to verify that both systems obtained high percentages of oxidability decrease. For the soil system there was a reduction of 72% and for the soil-biomass system there was a reduction of 70% (no statistically significant differences, p >0.0.5). Moreover, the spring variety of *C. sativa* obtained a smaller reduction of oxidability, namely for WW1 (no statistically significant differences).

The results are in line with the study of Ayaz *et al.*, (2001), where it was shown that different plant species (*Phragmites*, *Cyperus*, *Rush*, *Iris*, *Lolium*, *Canna*, and *Paspalum*) were able to depurate domestic wastewaters with approximately 88% removal of COD. Namely, *Iris* had the best COD removal in the order of 94%. Another study by Ferrara., (2013), demonstrated the phytodepuration capacity of *Cyperus papyrus*. This species was able to decrease the initial COD value of the wastewater (manufacture of mozzarella) by 98%.

However, the results don't confirm the presence of a synergistic effect between soil, microorganisms (present in the roots) and plants reported by O'Brien *et al.*, (2017). As there aren't significant differences between the soil and the soil-biomass systems, isn't possible to confirm this synergistic effect that promotes the efficiency of depuration of contaminants present in wastewaters. This may be due to the reduced volume of *Camelina sativa* roots (less than 1%), unlike other crops such as perennial crops. These crops have a greater distribution of the root fraction, having 50% of the soil volume (at the most superficial level, 0-20 cm).

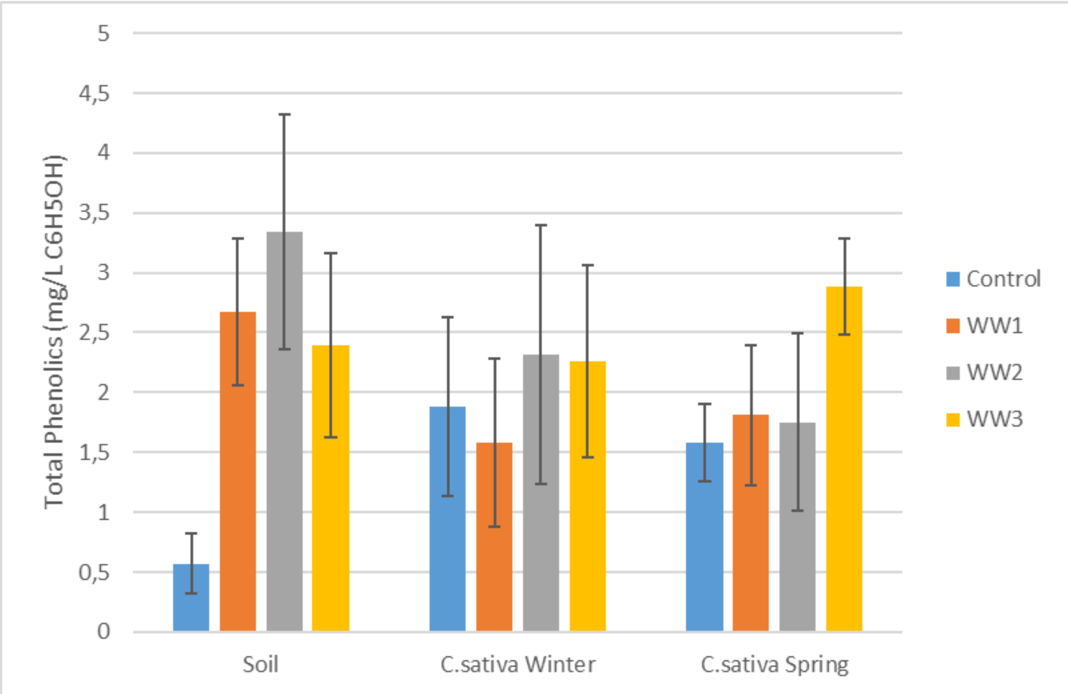

Figure 7.6- Average total phenolics values (mg/L C_6H_5OH) from the percolated waters of the soil, spring and winter varieties of *C. sativa*.

Figure 7.6 shows that there were quite a few oscillations of phenolics values in the soil and in soil-biomass systems, but not statistically significant (p >0.0.5). The results obtained in these two systems were lower than the initial phenolic values of the HTC effluent (34 ± 10 mg/L C_6H_5OH). This decrease in phenolics may be due to the oxidation of this organic compound to air exposure (Delvin *et a al*.,1984), but the soil and the soil-biomass can also have a task in this reduction.

Moreover, soil-biomass system has lower values of phenolics, this may suggest that *C. sativa* improves the capacity to depurate the phenolics present in the different wastewaters. Studies on phytoremediation of contaminated effluents have shown that endophytic bacteria, which are non-pathogenic bacteria within plant hosts, and rhizospheric bacteria present in the plant roots have the ability to degrade toxic compounds present in the contaminated effluents, including phenolics (González *et al*., 2013).

Another study by Yamaga *et al*., (2010) identified and isolated a bacterium *Acinetobacter calco-aceticus* P23 present in the rhizosphere of duckweed *Lemna aoukikusa*. This study demonstrated that there is a symbiotic relationship between the plant and the bacteria, promoting plant's growth and the constant removal of phenols in the culture medium. Thus, it's possible that *Camelina sativa* has some specific bacteria present in its roots that promotes the degradation of phenolics present in the wastewaters and consequently allows *C. sativa* to have phytodepuration capacity

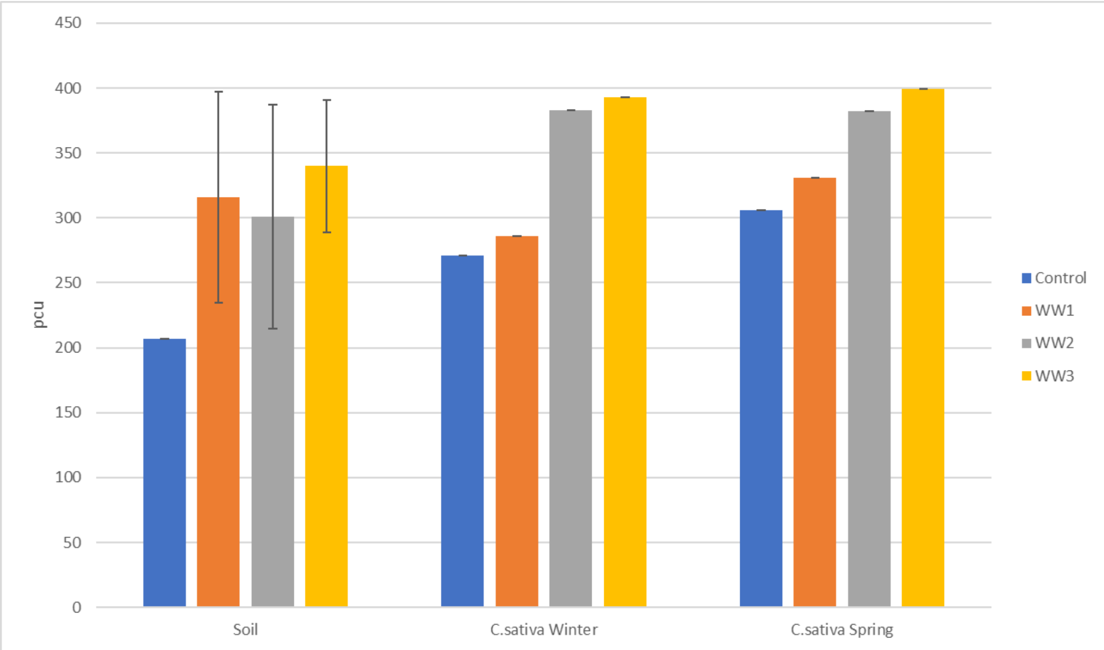

Figure 7.7- Average values of color analysis (pcu) of the percolated waters for January and May.

From the figure 7.7 it's possible to verify, that the color of the percolated waters from the months of January and May vary depending on the irrigation of the different wastewaters.

The soil system and the soil-biomass system (winter and spring varieties) have slightly different patterns. For the soil system there is a clear difference between the control and the pots irrigated with wastewaters. In the case of the pots with *Camelina sativa*, the color of the percolated waters obtained with the WW1 was comparable to those obtained in the control pots and lighter than the percolated waters obtained in the WW2 and WW3. This is an expected result, as these wastewaters have a higher load of organic and inorganic compounds.

In addition, both systems were efficient in reducing the initial value of color of the HTC effluent (3190 pcu). However, *Camelina sativa* may have influenced the color of the percolated waters, since the soil-biomass system has darker color than the percolated waters from the soil system.

This may be due to the combination of different factors, such as the availability of *C. sativa* to depurate the different organic and inorganic compounds present in different wastewaters. As well, the particle size differences present in the wastewaters (Farraji *et al*., 2017).

As mentioned above, due to the absence of significant differences between the results of soil and soil- biomass systems, it isn't possible to confirm the synergistic effect between soil, microorganisms (present in the roots) and plants, that allows a better depuration capacity (O'Brien et *al*.,2017).

7.2.1 Analysis of percolated waters and HTC effluent by GC/MS

The HTC effluent contains different oxygenated and aromatic compounds formed during the HTC process and sufficiently polar to be dissolved in the process water instead of adsorbed to the carbonaceous products of HTC. Those compounds include organic acids, phenolic derivatives and their chromatographic profile is presented in figure 7.8.

The peaks with higher chromatographic areas are eluted at earlier retention times indicating that they have lower molecular weight. This is an expected behavior because the HTC byproducts with higher molecular weight are probably less polar, so they were predominantly adsorbed in the hydrochar instead of dissolved in the process water (Due *et al*.,2018).

The chromatographic profiles of the percolated waters of May, corresponding to the soil system and the soil-biomass system (winter variety) irrigated with WW2, are presented in figures 7.9 and 7.10. The chromatograms for the remaining January and May percolated water samples are in the Appendices (Figure A.1-A.13)

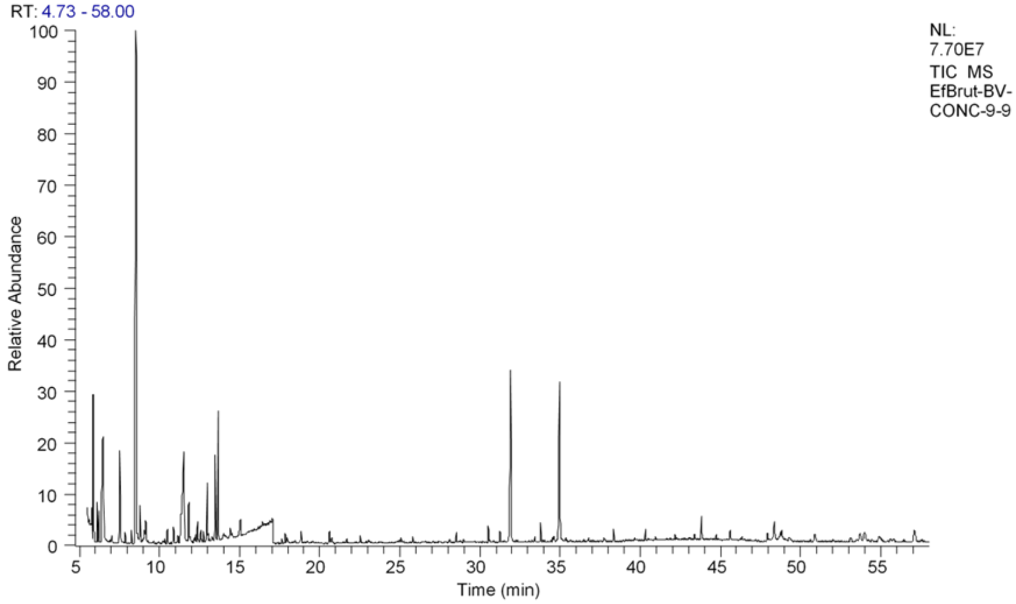

Figure 7.8-- Chromatographic profile of the organic compounds detected in the HTC effluent.

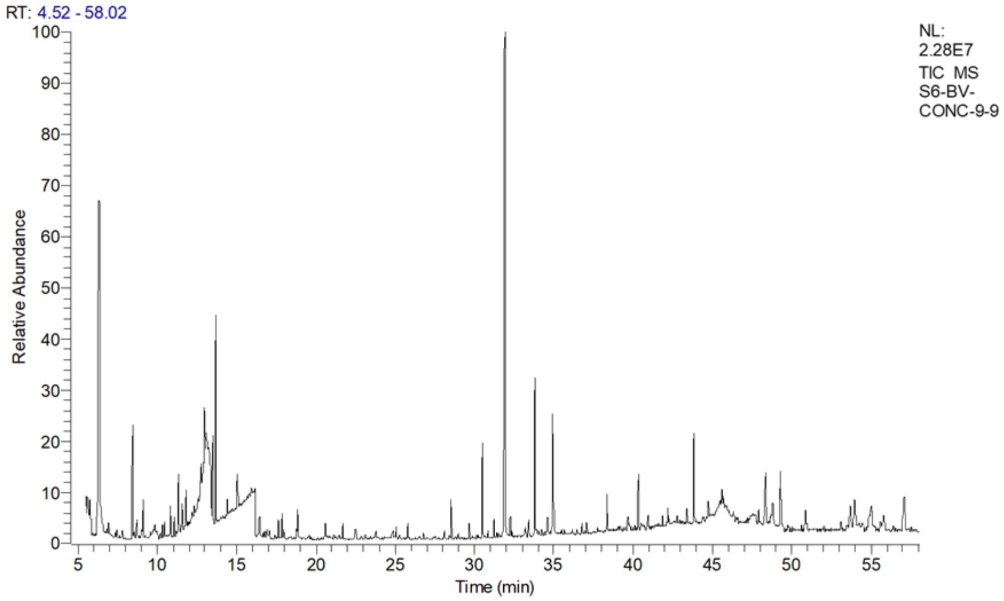

Figure 7.9- Chromatographic profile of the organic compounds detected in the percolated water (WW2) of May in a soil system.

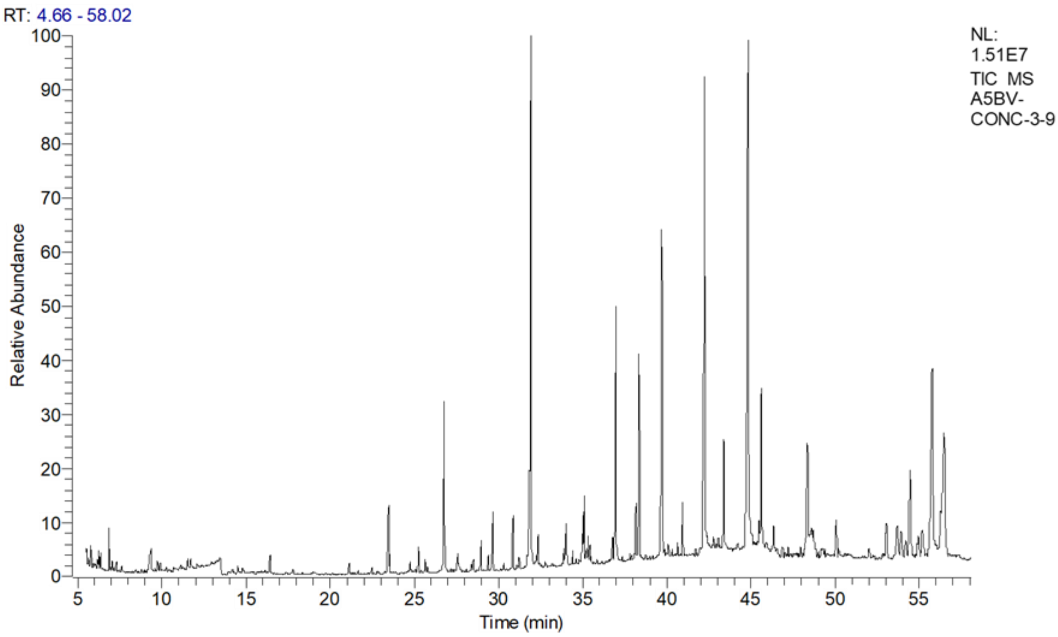

Figure 7.10- Chromatographic profile of the organic compounds detected in the percolated water (WW2) of May in a soil-biomass system (*C. sativa*, winter variety).

When comparing with the HTC profile is noticeable the appearance of different organic compounds, typically for retention times higher than 25 min. These organic components were not detected in the HTC effluent, therefore must result from dissolution of soil or biomass components during percolation of the wastewaters. Furthermore, for the soil-biomass system there was a clear reduction of organic components eluted before 20 min that were abundant in the HTC effluents.

To compare the abundance and concentrations of the organic components detected in the HTC effluent and in the percolated waters, the chromatographic areas of the compounds eluted in successive retention time windows with 5 min bandwidth were added and the corresponding total areas were plotted as a function of the retention time window for the different samples analyzed (Figures 7.11 to 7.12).

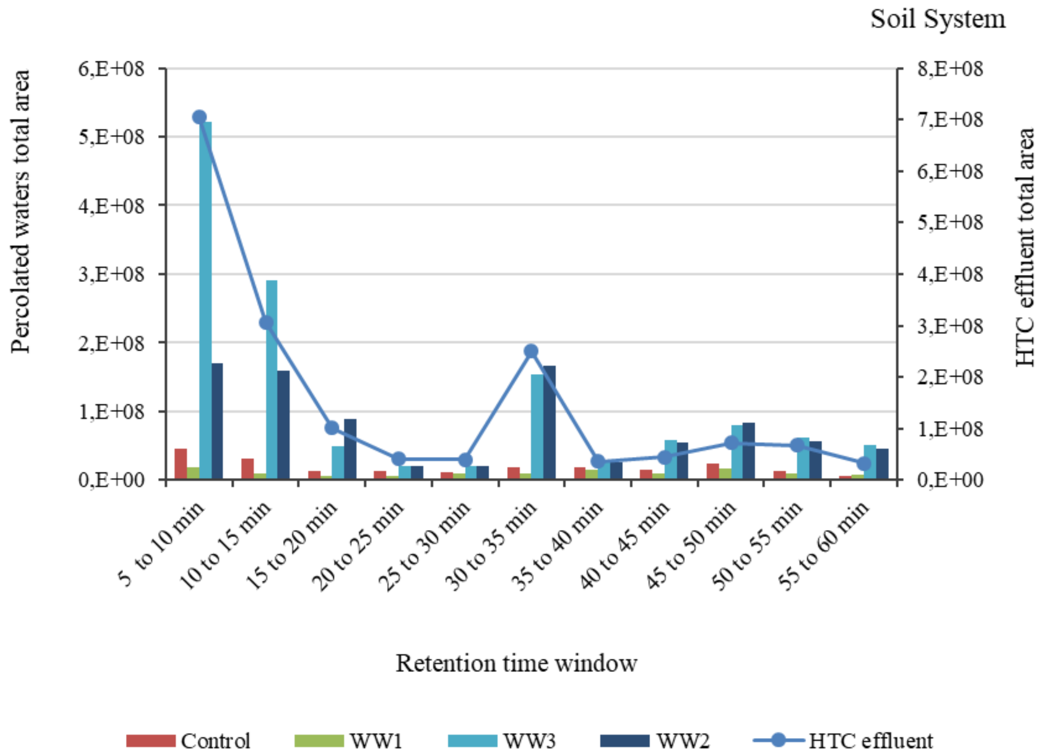

Figure 7.11- Sum of the peak chromatographic areas of the organic compounds detected in percolated waters and HTC effluent, as a function of their retention time window, for the soil system (May sample).

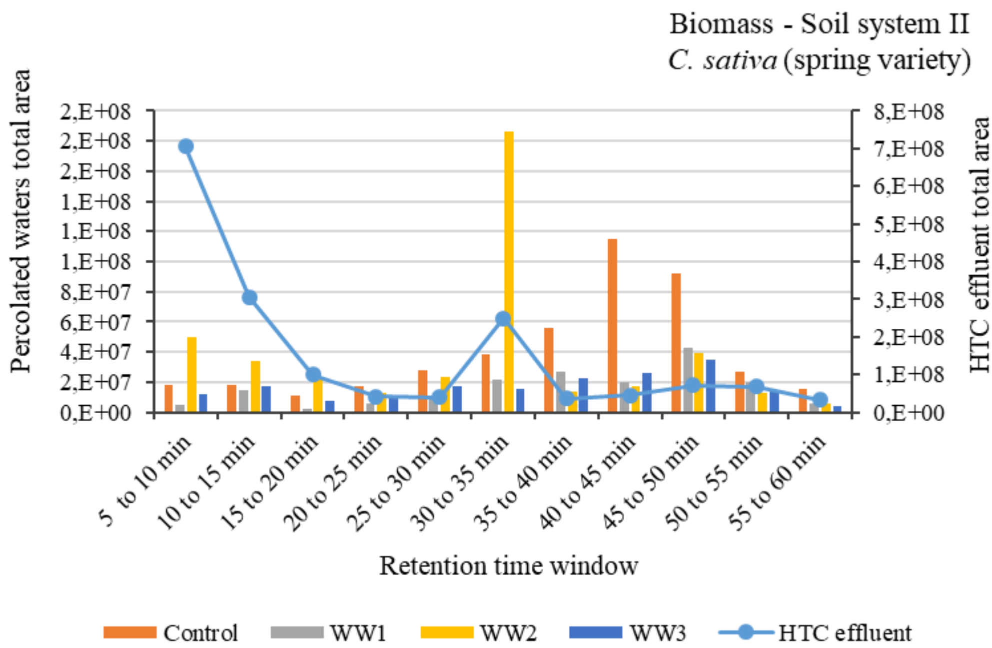

Figure 7.12- Sum of the peak chromatographic areas of the organic compounds detected in percolated waters and HTC effluent, as a function of their retention time window, for the soil-biomass system with *C. sativa*, spring variety (May sample).

The soil had the capacity to adsorb a fraction of the organic compounds present on the HTC effluent, especially for the less concentrated wastewaters. For retention times higher than 40 min, the percolated waters showed the presence of components not present in the HTC effluent, therefore leached from the soil.

Both soil and soil-biomass systems showed a better capacity to retain the organic compounds with retention times lower than 30 min that were present in the HTC effluent in higher concentrations. This effect was observed for the three concentrations of wastewaters (WW1, WW2 and WW3), so it wasn't dependent of the organic compound's concentration. Table 7.3 shows the removal rate of organic compounds by the soil and soil-biomass systems.

Table 7. 3- Removal rate of organic compounds for the soil system (SS), the soil-biomass system with the winter variety of *C. sativa* (CSw) and for the soil-biomass system with the spring variety of *C. sativa* (CSs), irrigated with wastewaters WW1, WW2 and WW3 (May samples).

Retention time window	**Removal rate (%)**								
	WW1-SS	**WW2-SS**	**WW3-SS**	**WW1-CSw**	**WW2-CSw**	**WW3-CSw**	**WW1 CSs**	**WW2-CSs**	**WW3-CSs**
5 to 10 min	97.4	76.0	26.1	98.6	97.1	97.6	99.3	93.0	98.3
10 to 15 min	96.7	48.3	4.8	89.3	94.3	89.9	95.1	89.0	94.3
15 to 20 min	94.7	11.5	50.5	76.9	93.7	90.2	97.5	78.3	92.1
20 to 25 min	86.6	50.0	49.0	77.9	61.9	68.5	85.4	69.3	74.6
25 to 30 min	77.1	50.9	49.0	74.5	8.7	74.8	78.1	41.8	57.2
30 to 35 min	95.8	33.5	38.2	32.4	65.7	59.3	91.4	25.6	93.6
35 to 40 min	59.3	30.7	26.2	53.0	-192.5	41.1	23.9	61.9	36.1
40 to 45 min	79.6	-22.4	-32.0	58.5	-303.5	4.8	55.5	61.4	42.3
45 to 50 min	77.2	-16.4	-10.6	54.6	-16.9	-8.4	41.0	45.4	50.9
50 to 55 min	84.9	17.7	7.2	69.6	7.2	8.3	70.7	80.5	79.2
55 to 60 min	78.2	-39.7	-54.5	72.3	-195.6	10.1	83.0	81.0	86.5

7.3 Characterization of biomass

7.3.1 Morphological parameters

7.3.1.1 Stems height

Regarding the morphological parameters of the collected biomass, the height of the spring and winter varieties of *Camelina Sativa* was evaluated. Stem height analysis of these two varieties allows to evaluate the plant growth capacity under the irrigation conditions of this study.

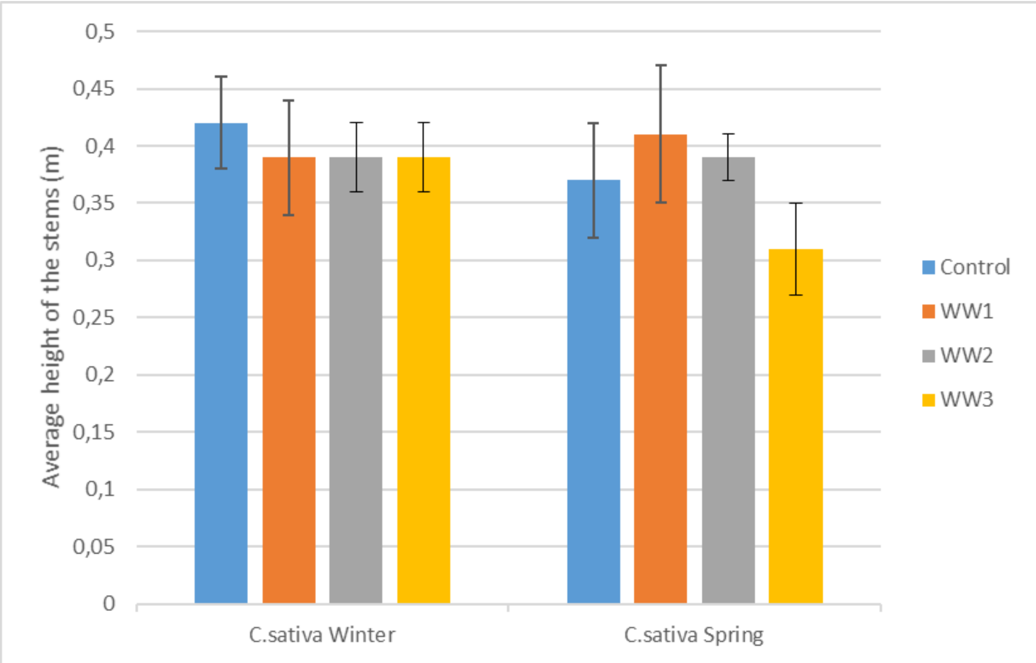

Figure 7.13- Average height of the stems of the winter and spring varieties of *C .sativa* under different irrigation with different wastewaters (WW1,WW2,WW3) and control.

Based on the results obtained, it can be observed that the spring variety of *C. sativa* presents more height variances between the different wastewaters and with the control (Figure 7.13). The values corresponding to the control has an average height of 0.37 m, compared to 0.41 m of WW1, 0.39 m of WW2 and 0.31 m of WW3. For instance, irrigation with WW1 has the highest value, as well as, the most variance in heights between the stem's samples of this wastewater group. This may be because wastewater WW1 is the most diluted water, and therefore doesn´t limit the growth of the spring variety of *C. sativa*. In contrast, the spring variety when irrigated with WW3 has the lowest average height value. This may suggest that *C. sativa* summer is more sensitive to less dilute wastewater (WW3), which limited the growth of the stems.

On the other hand, the winter variety of *C. sativa* presents homogeneous height values of 0.39 m when irrigated by the different wastewaters. This value compared to the average height of the control (0.42 m) shows that there is a decrease in height when the winter variety is irrigated by the wastewaters (not statistically significant; p>0.05). This suggests that the winter variety is less sensitive to wastewater irrigation compared to the spring variety of *Camelina sativa*. Although there aren't references in the literature of height differences between varieties, this differences may be due to genetic variability of the spring variant that makes it more sensitive to contaminants that are present in the wastewaters (Obeng *et al.*,2019). The heights obtained from the two variants of *Camelina sativa* are much smaller than those obtained in the study of Anderson *et al*. (2018). This study obtained 0.70 m of height for the spring variety, and about 0.65 m of height for the winter variety. This reduction in height may also be due to the use of small pots, which compromised the growth of the *C. sativa* root system (Wittenberg *et al*.,2019).

7.3.2 Productivity

Productivity is a parameter that is related with the growth and development of the plant and consequently to its economic valorization. The following figures 7.14,7.15 and 7.16 shows the yield results of the various plant structures of the two *Camelina sativa* varieties.

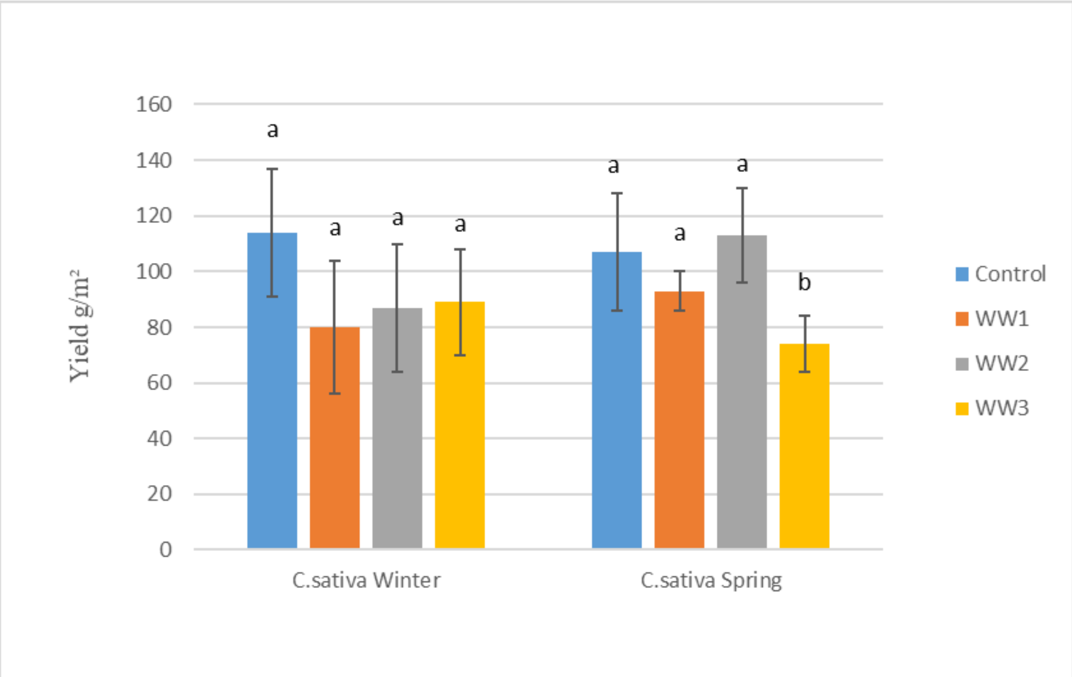

Figure 7.14- Average aboveground yield (g/m2, dry matter) under different irrigation with different wastewaters (WW1, WW2 and WW3) and control. (a-b, different subscript letters, for the same variety, means statistical significant differences (p<0.05)

Figure 7.14 presents the aboveground yield which is constituted by the leaves, siliquae and the stems. Regarding the analysis of this figure, it's possible to observe that the yield corresponding to the winter variety control (114 g/m2) stands out to the remaining wastewaters yields, yet the differences aren't statistically significant. In contrast, the spring variety has a maximum yield value of 113 g/m2 corresponding to WW2, which is higher than the control yield (107 g/m2). This variety have a yield loss when irrigated with WW1 (not statistically significant) and WW3 (statistically significant when compared with the control). Therefore, it can be suggested that the wastewaters (WW1, WW2 and WW3) affect the plant yield, since the difference between the control yield, of the two varieties, and the wastewaters yield values show a reducing trend, which was more significant for the spring variety and for the plants obtained from the pots irrigated with WW3.

In this view to have a more detailed analysis of *Camelina sativa* yield, the average seed and root yield were also evaluated. The Figure 7.15 and 7.16 presents the siliquae and root yield, respectively.

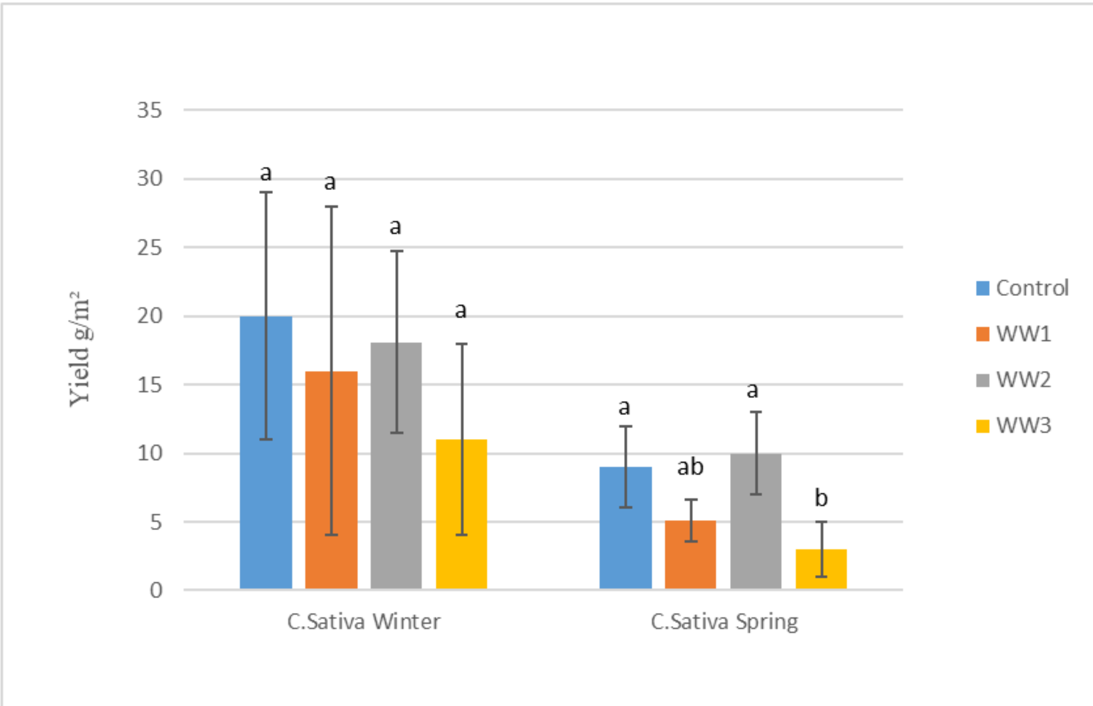

Figure 7.15- Average siliquae yield (g/m^2, dry matter) under different irrigation with different wastewaters (WW1, WW2 and WW3) and control.

Figure 7.15 shows a major difference of seed yield between the two varieties of *Camelina sativa.* In the first place, the spring variety presents a much lower yield compared to the yield obtained in the winter variety. This reduction in siliquae yield may be due to unfavorable weather as verified by Zubr., (1997). Furthermore, this difference may also have been affected by several factors such as year of cultivation, site, cultivar and differences in nutrient uptake due to genetic variability of the spring variety of *C.sativa* (Urbaniak *et al*., 2008) (Obeng *et al*.,2019).

The highest yield value corresponds to WW2 (10 g/m2), which is in line with the results obtained in the aboveground yield. In comparison, the winter variety had higher seed yield (control, WW1 and WW2) than the spring variety. Although, WW3 irrigation caused a 50% reduction of seed yield (without statistical significance).

More important, it seems that there is a tendency for siliquae yield to decrease when *C. sativa* is irrigated with the most polluting wastewater (WW3), particularly for the spring variety (with significant statistical differences). This decrease may affect seed and oil production, which consequently damage *C. sativa* oil productivity and its economic viability may be compromised.

This is in conformity to what was mentioned above that the wastewaters don´t influence positively the yield. Chatzakis *et al* (2011) also observed a decrease in seed yield of sunflower (*Helianthus annus L*.) and castor (*Ricinus communis L.)* seeds when irrigated with municipal wastewater effluent in comparison with freshwater irrigation. The researchers suggested that this decrease was due to the level of soil salinity present in effluent irrigated pots. This may also have happened in the soil-biomass system (winter and spring variants of *C. sativa*).

In addition to the above, the presence of organic acids and phenols from the HTC effluent may have also affected the growth of *Camelina sativa* and consequently affected the siliquae yield (Kuiters,.1989) (Tunes *et al*.,2012).

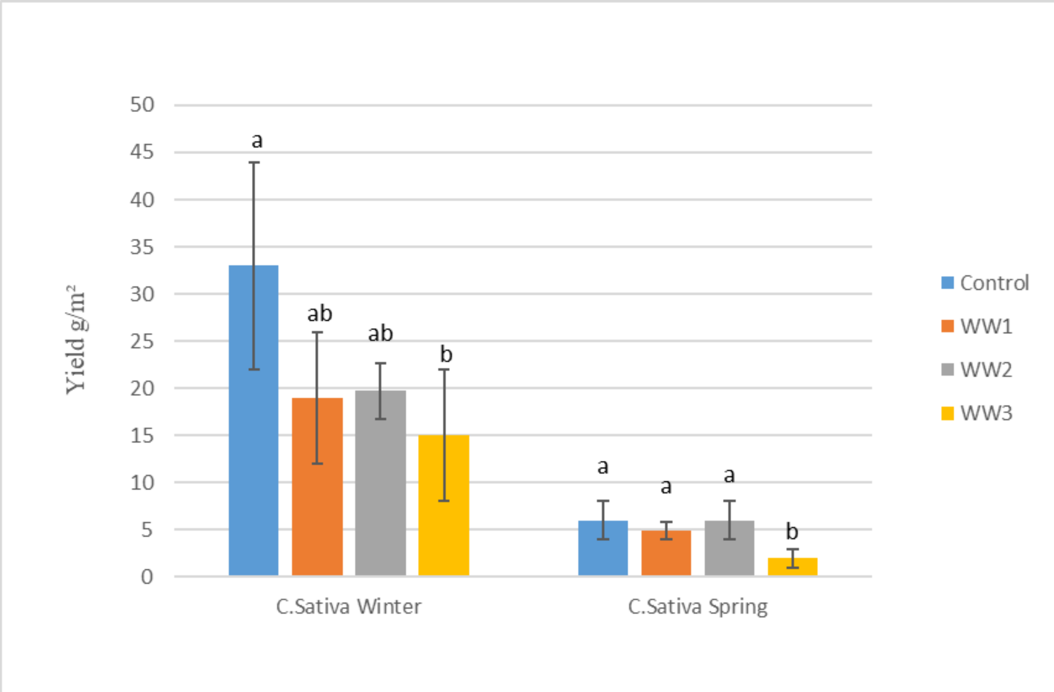

Figure 7.16- Average root yield (g/m^2, dry matter) under different irrigation with different wastewaters (WW1, WW2, WW3) and control. (a-b, different subscript letters, for the same variety, means statistical significant differences ($p<0.05$).

Regarding the root yield, it´s possible to verify that the spring variety of *C. sativa* has a much lower yield comparing to the winter variety (Figure 7.16). In the spring variety, the yield values of the control, WW1 and of the WW2 are the same (5-6 g/m2), which suggests that the roots yield weren´t influenced by the wastewaters. However, in pots irrigated with WW3, the yield loss was significant compared with control and WW1-2. The same happened with the winter variety where results indicate that irrigation with the highest contaminated wastewater induced a yield reduction.

The phenolics from the HTC effluent may have affected the growth of the root system and consequently root yield (Kuiters,.1989). Berti *et al*. (2011) refers that *Camelina sativa* when produced in the Mediterranean area has a condition, which is not being able to withstand well temperatures above 25ºC and consequently doesn´t develop at its maximum temperatures. In view of this condition, the spring variety of *Camelina sativa* was sown during the winter.

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7.3.3 Chemical parameters

7.3.3.1 Ash content

This chemical parameter is very important for energy production, because the ash content present in the different types of biomass can cause numerous problems. These problems can be the formation of deposits on surfaces exposed to radiant heat due to the alkaline elements, corrosion of metal surfaces and the formation of deposits on heat-recovery surfaces (Cassida *et al*., 2005). It's also shown that calorific heat is negatively related to ash content, that is, the higher the percentage of ash biomass, the less interesting the biomass will be for energy purposes (Monti *et al*.,2008).

The results corresponding to the average ash content analysis of the different structures of *Camelina sativa* are presented in the figures 7.17, 7.18 and 7.19.

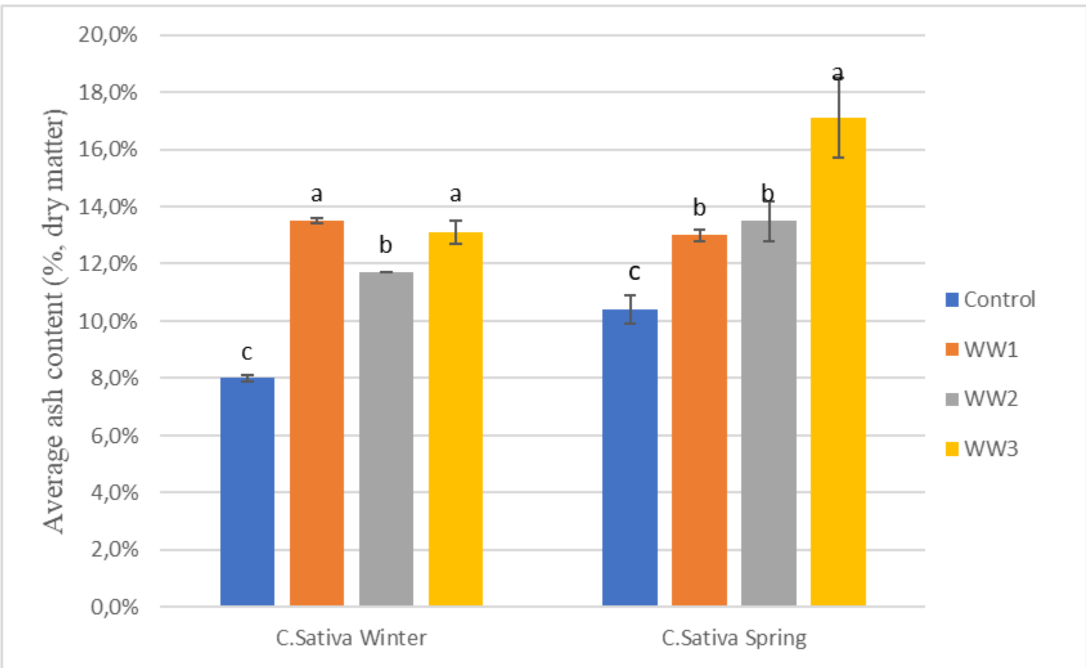

Figure 7.17- Average ash content (%, dry matter) in steams under different irrigation with different wastewaters (WW1, WW2, WW3) and control. (a-b, different subscript letters, for the same variety, means statistical significant differences ($p<0.05$).

From figure 7.17 it´s possible to verify that the spring variety of *C. sativa* has the highest ash content value, which corresponds to WW3 (17.1%, dry matter). In this variety, the plants irrigated with the different wastewaters (WW1, WW2 and WW3) have higher ash content compared to the control (10,4%, dry matter). Equally, the winter variety have higher wastewaters values than the control (13,5% for WW1, 11,7% for WW2 and 13,1% for WW3).

It can be observed that with the increase of wastewater pollution load there is also an increase of ash content. This phenomenon is verified in the two varieties of *Camelina sativa*, because possibly the wastewater has ions and salts that as its concentration increases, the plant accumulates in its composition (Rozema *et al*., 2014). The high ash content of the spring variant may be due to salt and ion accumulation, as well as, high concentration due to low productivity.

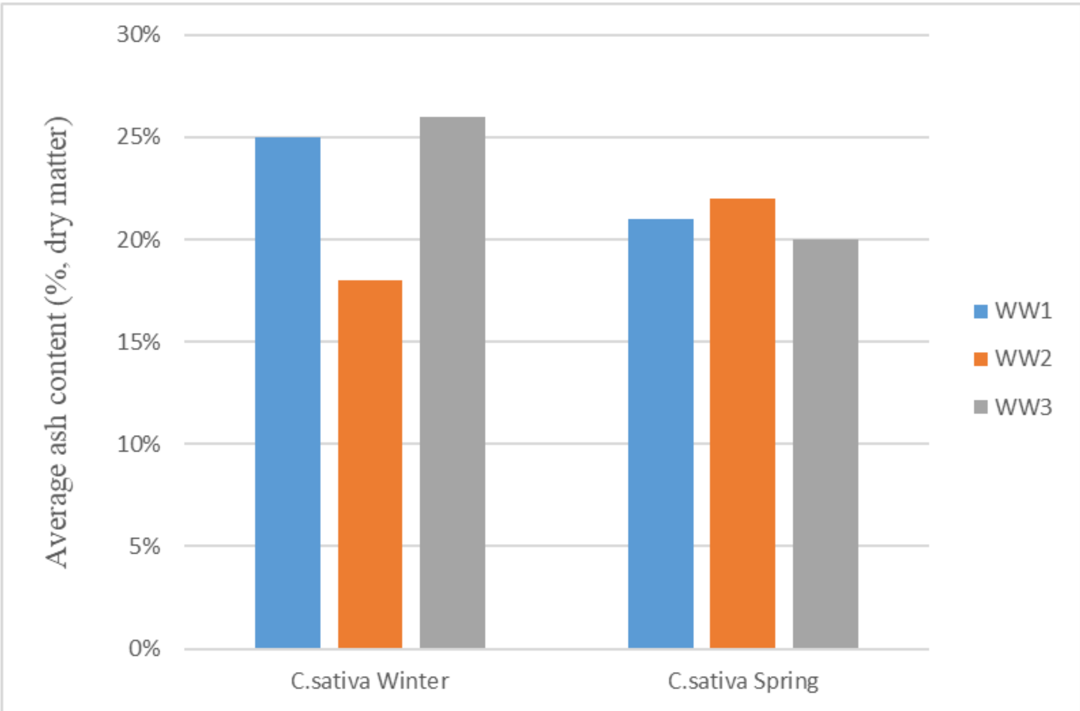

Figure 7.18-- Average ash content (% dry matter) in leaves submitted to different irrigation with different wastewater (WW1, WW2, and WW3) and control.

In the analysis of the leaves, at the time of the harvest, there weren't more leaves in the control pots. Therefore, it isn't possible to compare the ash content of leaves obtained from pots irrigated with WW and pots irrigated with tap water. Results presented, show, nevertheless that there were no differences in terms of ash content among the different types of WW's. And, between the two varieties (Figure 7.18).

From the leaf analysis, it´s possible to verify that it´s the plant fraction that has the highest percentage of ash content. The winter variety of *C. sativa* has the most variances, with WW3 having the highest value of 26% dry matter. Regarding the spring variety, the values didn´t show oscillations, with the highest value being 22% dry matter for WW2 and the lowest value of ash content was 20% dry matter for WW3.

This is an expected result, since the leaves are the crops structure with the highest ash content, for most of the crops. As Monti *et al*, (2008) refers, the leaves have 50% more ash content than the other fractions of biomass. The high ash content may be due to plant metabolism in nutrient accumulation (Vamvuka *et al.*, 2011).

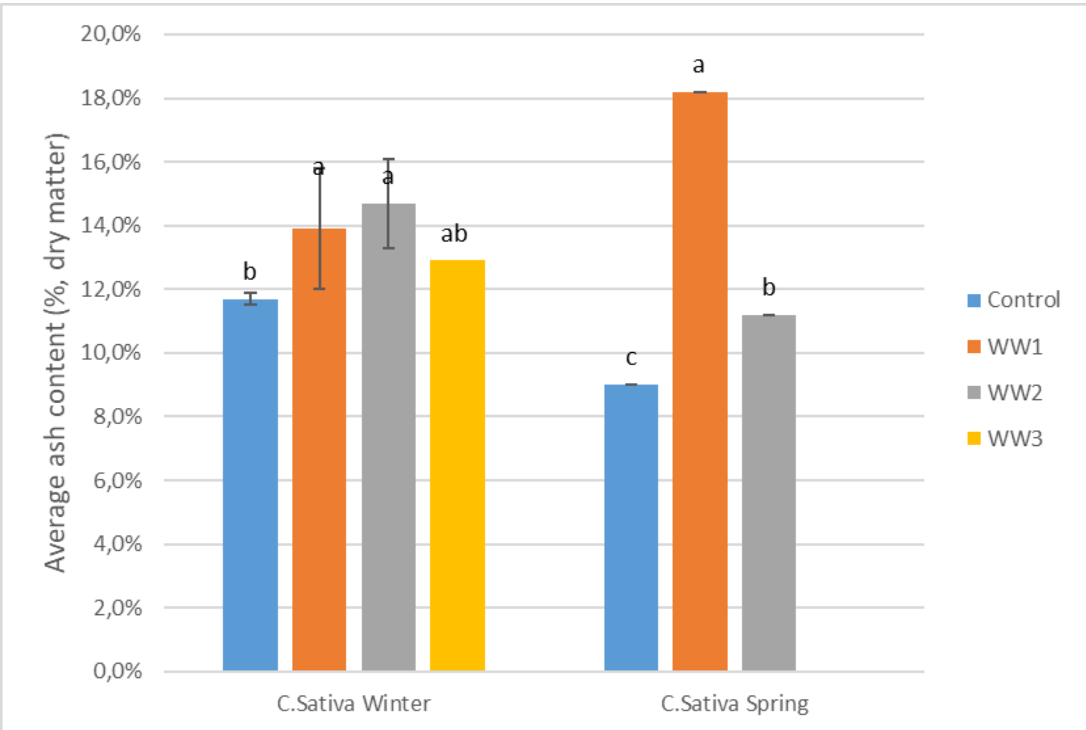

Figure 7.19- Average ash content (% dry matter) in siliquae submitted to different irrigation with different wastewater (WW1, WW2 and WW3) and control. (a-b, different subscript letters, for the same variety, means statistical significant differences (p<0.05).

The ash content analysis in the siliquae shows that the spring variety of *C. sativa* presents high oscillations between the different wastewaters (Figure 7.19). In fact, WW1 has the highest value of 18,2% ash content compared with the control (9,0%). The amount of sample of the spring *Camelina sativa* obtained in pots irrigated with WW3 was not enough to proceed with analysis of ash content. In contrast, the winter variety has milder oscillations having the highest value corresponding to WW2 (14.7 %, dry matter). Yet, results indicate a trend to a higher ash content when pots are irrigated with WW's. This may compromise the amount of oil in the siliquae and therefore the economical exploitation of the crop.

Ash content of the roots was 16 ± 6 % (dry matter), and no significant differences were observed due to the different wastewaters and the different varieties.

To conclude, the leaves have more ash accumulation as they have the highest ash content value. At the same time, the roots and siliquae were the second structures of *Camelina sativa* that presents more ash accumulation, may affect *C. sativa* oil quality.

7.3.3.2 Nitrogen content

The analysis of nitrogen content in biomass for energy production is of utmost importance as a large percentage of nitrogen in the biomass may lead to increased NO_x emissions during biomass combustion as also when biomass is used in other thermochemical processes. Consequently, NO_x emission contributes to the release of environmentally harmful gases and acidification. (Gomes *et al*.,2018)

The results of average nitrogen content analysis of the different plant structures under different irrigation regimes are shown in the figures 7.20, 7.21 and 7.22. It´s possible to observe that in this analysis the higher accumulation of nitrogen was observed in the siliquae and stems and no significant differences were observed between the two varieties.

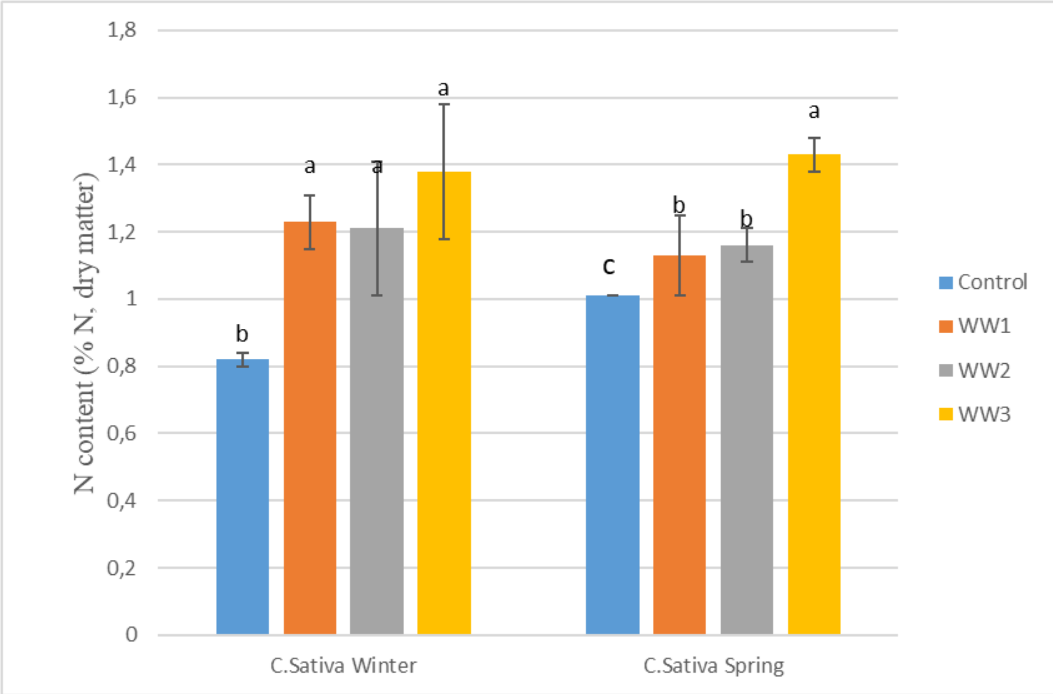

Figure 7.20- Average nitrogen content of C. sativa stems (% N, dry matter) for different irrigation with different wastewaters (WW1, WW2 and WW3) and control(a-b, different sub-script letters, for the same variety, means statistical significant differences ($p<0.05$).

The figure 7.20 shows that both *C. sativa* varieties have oscillations of nitrogen content between the different wastewaters and the control. The highest values were obtained by WW3 in both varieties, having maximum value of 1,38 (%N, dry matter) for the winter variety and value of 1,43 (%N, dry matter) for the spring variety of *C. sativa*. As can be seen from the results, there is indeed an influenced by wastewaters, namely WW3, on the accumulation and increase of nitrogen content in the plant stems compared to the control.

This increase of nitrogen concentration may be due to lower yields, since HTC effluent has very low nitrogen values. In addition, the increment may compromise the use of the stems in the thermochemical processes, but if the stems are left in the ground, this will enrich the soil in nitrogen, which is beneficial for the soil. The same pattern was observed in the roots. The winter variety showed lower concentration of nitrogen than the spring variety once the yields were higher. And for both varieties, the N content increased with the load of wastewater, due to the yield reduction. Winter variety showed a range of 0.58 (control) to 0.99 % N (irrigated with wastewaters), and the spring variety showed a range of 0.85 (control) to 1.57 % N (irrigated with wastewaters).

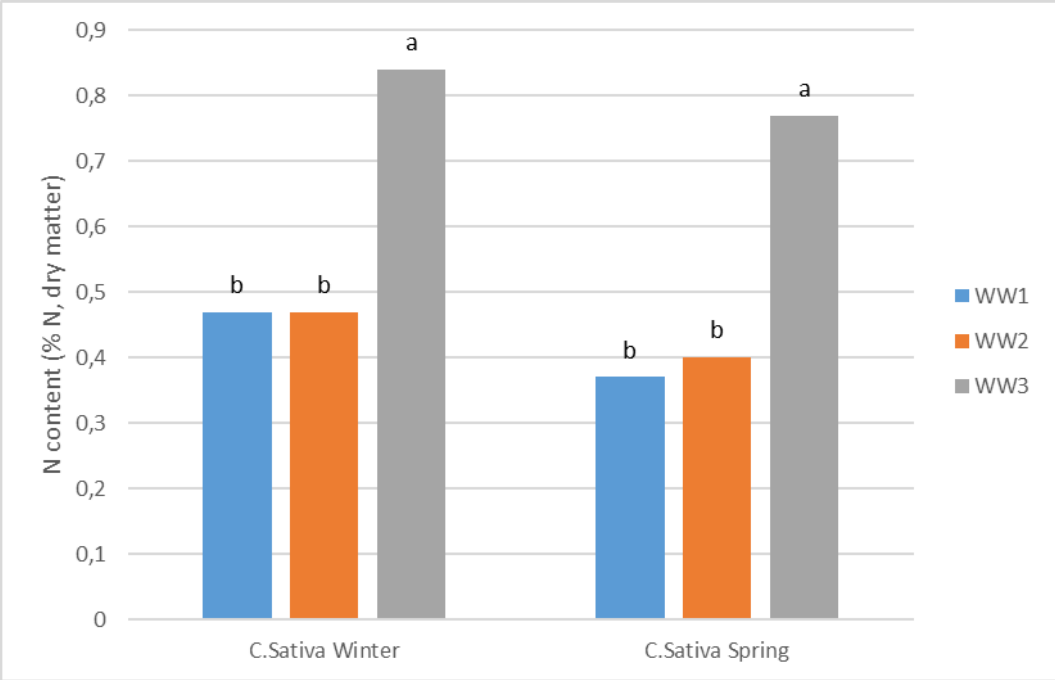

Figure 7.21- Average nitrogen content of C. sativa leaves (% N, dry matter) for different irrigation with different wastewaters (WW1, WW2 and WW3) and control (a-b, different subscript letters, for the same variety, means statistical significant differences ($p<0.05$).

The figure 7.21 shows that weren´t any leaves obtained from the control irrigation. However, it´s possible to observe that there is a major difference in nitrogen content values for both varieties of *Camelina sativa*. These differences correspond to wastewater WW3, which have a nitrogen content value of 0,84, %N, dry matter for the winter variety and a value of 0,77%N, dry matter for the spring variety. Overall, these results are in line with the results obtained from the stems, since once again the wastewater WW3 influenced and increased the nitrogen content in both varieties of *C. sativa*. In this case, leaves are usually left in the ground during harvest and thus, this nitrogen can return to the soil enriching the soil.

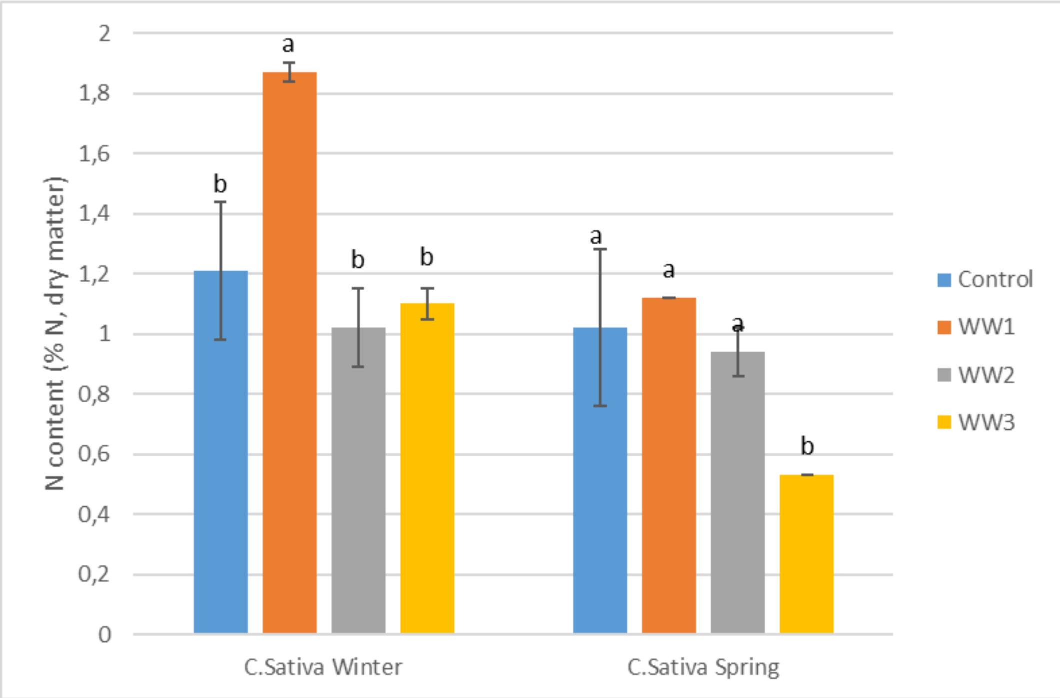

Figure 7.22- Average nitrogen content of *C. sativa* siliquae (% N, dry matter) for different irrigation with different wastewaters (WW1, WW2 and WW3) and control. (a-b, different subscript letters, for the same variety, means statistical significant differences ($p<0.05$).

The winter variety has nitrogen content oscillations (Figure 7.22), with WW1 having the highest value of 1,87 (%N dry matter) compared to the other wastewaters and to the control (1,21, %N dry matter). Although the spring variety has lower nitrogen content values, WW1 has the highest value in this variety (1,12, %N dry matter). However, globally, results seem to indicate that nitrogen content in the siliquae was not affected by the irrigation with wastewaters.

7.3.3.3 Phosphorus content

Phosphorus is very important for the plant as it's an essential component of adenosine triphosphate (ATP). ATP is vital for many biochemical processes such as plant development, uptake of various nutrients, as well as their transport through the plant. In addition, phosphorus is also an essential component for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) that are closely related to protein synthesis. Therefore, for the plant to grow optimally, phosphorus concentration must be 0,3-0,5 (%P, dry matter) (Mishra *et al*.,2010).

The results obtained corresponding to the average phosphorus content present in the different structures of *Camelina sativa* are presented in the figures 7.23, 7.24 and 7.25. It's possible to verify, as in the analysis of nitrogen content, the winter variety of *C. sativa* was more likely to accumulate phosphorus, particularly in the stems, siliquae and roots.

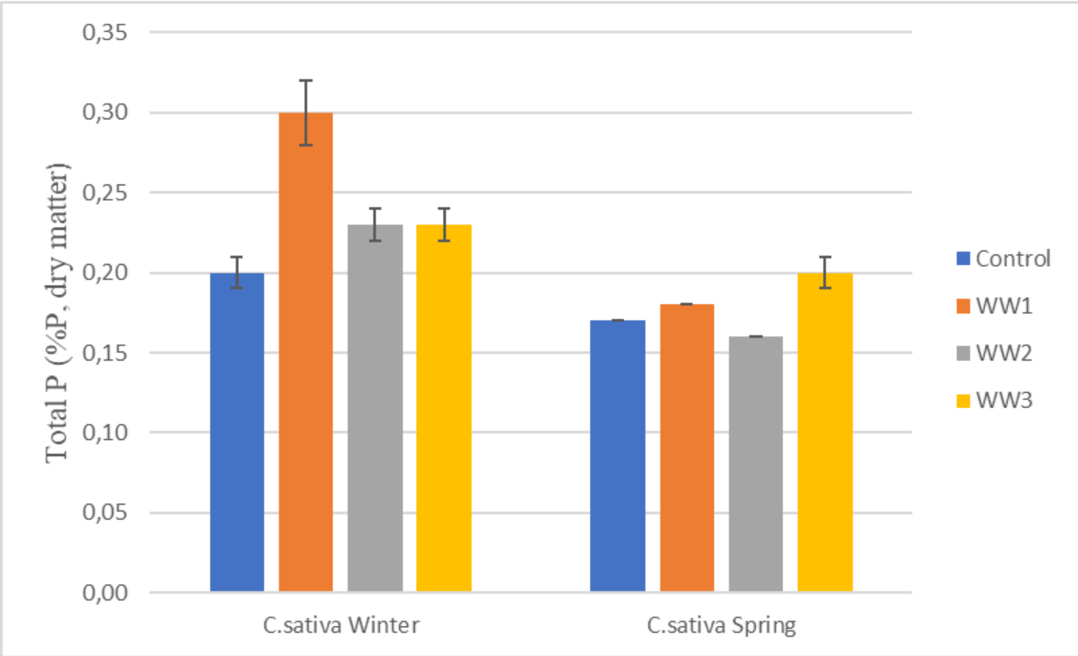

Figure 7.23- Average phosphorus content of *C. sativa* steams (% P, dry matter) for different irrigations with different wastewaters (WW1, WW2 and WW3) and control.

Through the analysis of figure 7.23 it's possible to observe that both plant varieties suffer phosphorus content oscillations, however the winter variety presents higher values. Namely, the value corresponding to WW1 is the highest (0,30, %P dry matter) compared to the remaining wastewaters and to the control (0,20, %P dry matter).

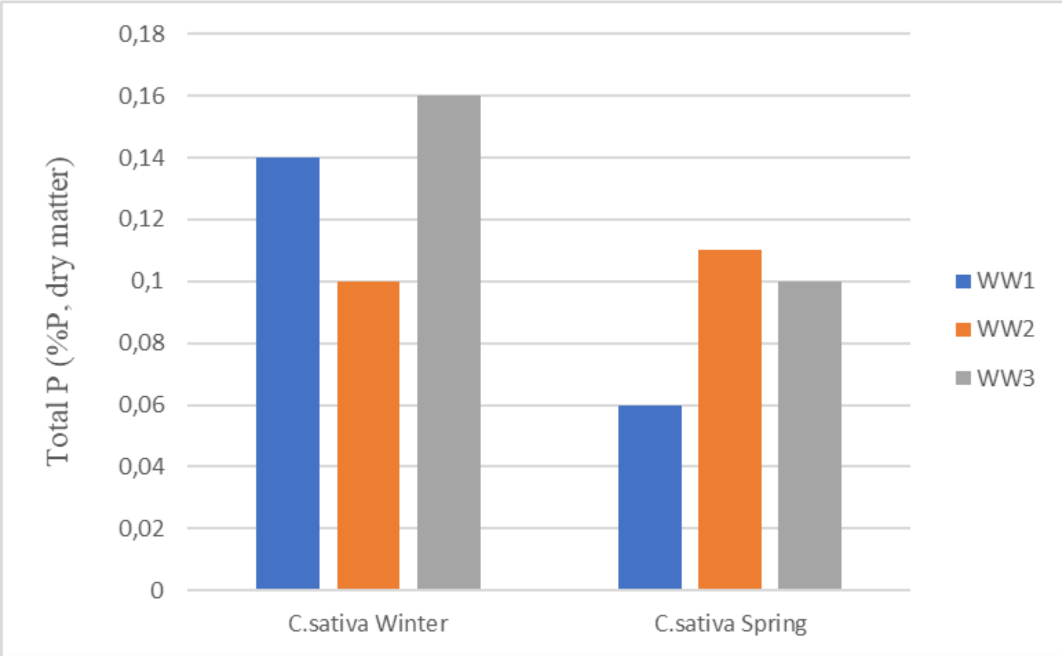

Figure 7.24-Average phosphorus content of *C. sativa* leaves (% P, dry matter) for different irrigations with different wastewaters (WW1, WW2 and WW3) and control.

Regarding the analysis of phosphorus content in the leaves, there weren´t any leaves obtained from the control plants (Figure 7.24). However, from the figure it's possible to observe that winter variety was more susceptible to the accumulation of phosphorus, having the highest value of 0,16 (%P, dry matter) corresponding to WW3. While the spring variety of *C. sativa* had the highest value of 0,11 (%P, dry matter) corresponding to WW2.

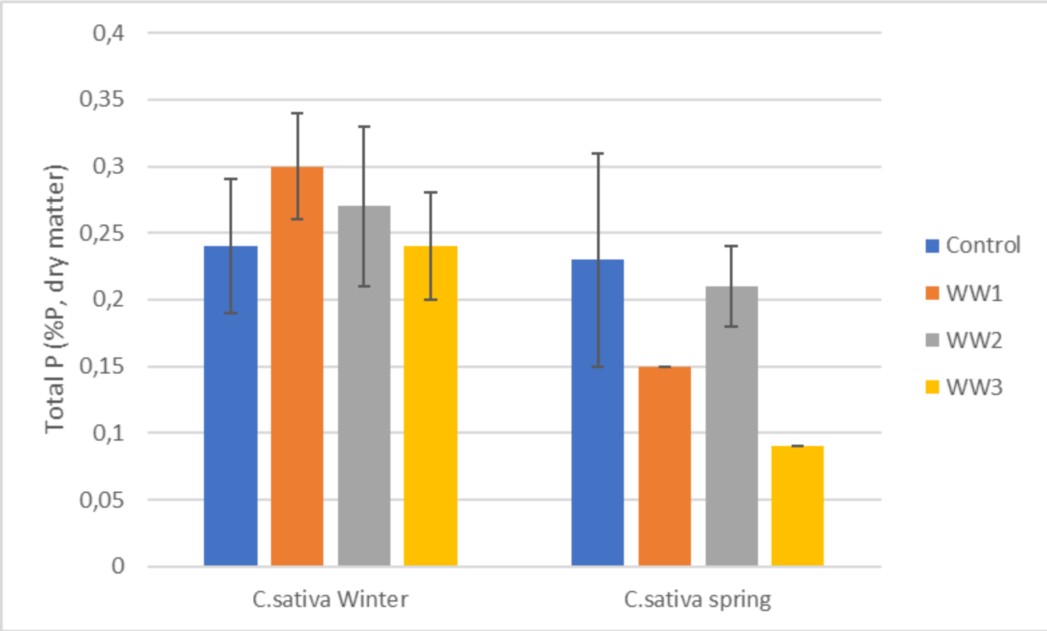

Figure 7.25- Average phosphorus content of *C. sativa* siliquae (% P, dry matter) for different irrigations with different wastewaters (WW1, WW2 and WW3) and control.

The phosphorus content in the siliquae shown in the figure 7.25 fluctuates greatly for both *Camelina sativa* varieties. The winter variety has higher values than the spring variety, with the maximum value of 0,30 (% P, dry mater) corresponding to WW1.While the spring variety has the highest value of 0,23 (%P, dry matter) corresponding to the control.

These results are in agreement with the results obtained in the analysis of the stems, since it's verified that the winter variety of *C. sativa* is more susceptible to accumulate phosphorus when irrigated with the wastewater WW1.These results are in agreement with the values stipulated by Mishra *et al*. (2010) for optimal plant growth. Phosphorus content of the roots was 0,31 ± 0.11 % (dry matter), and no significant differences were observed due to the different wastewaters and the different varieties. This fraction of the plant was the one that presented the highest concentration of P.

7.3.3.4 Metal content

Tables 7.4 and 7.5 shows the content of the biomass in terms of metals for different fractions of the biomass. Results represent an average, for each variety, among all the biomass collected, once no significant differences were observed among the different irrigation systems.

Table 7.4- Metals (Ca, Mg, Na, K) content of the different fractions of the biomass of *C. sativa*

	Plant fraction	Ca (% w/w)	Mg (% w/w)	Na (% w/w)	K (% w/w)
***C. sativa* winter**	Stems	0.6 ± 0.1	0.40 ± 0.03	0.06 ± 0.01	1.0 ± 0.1
	Leaves	0.9 ± 0.1	0.48 ± 0.02	0.07 ± 0.01	1.7 ± 0.2
	Siliquae	1.0 ± 0.1	0.50 ± 0.02	0.07 ± 0.02	1.5 ± 0.1
	Roots	1.1 ± 0.1	0.48 ± 0.02	0.08 ± 0.02	1.4 ± 0.1
***C. sativa* spring**	Stems	0.5 ± 0.1	0.38 ± 0.02	0.05 ± 0.02	1.0 ± 0.1
	Leaves	0.9 ± 0.1	0.62 ± 0.07	0.08 ± 0.01	1.5 ± 0.2
	Siliquae	0.9 ± 0.1	0.56 ± 0.03	0.09 ± 0.02	1.4 ± 0.2
	Roots	1.1 ± 0.1	0.48 ± 0.02	0.08 ± 0.02	1.4 ± 0.1

Table 7.5- Metals (Fe, Mn, Cu, Zn) content of the different fractions of the biomass of *C. sativa*

	Plant fraction	Fe (mg/kg w/w)	Mn (mg/kg w/w)	Cu (mg/kg w/w)	Zn (mg/kg w/w)
***C. sativa* winter**	Stems	147 ± 10	5.2 ± 0.1	11 ± 1	62 ± 8
	Leaves	184 ± 38	6.2 ± 0.7	11 ± 1	104 ± 27
	Siliquae	235 ± 66	8,1 ± 0.4	10 ± 2	54 ± 9
	Roots	1760 ± 122	8.0 ± 0.4	9.4 ± 0.4	108 ± 19
***C. sativa* spring**	Stems	184 ± 20	5.5 ± 0.2	12 ± 1	50 ± 13
	Leaves	174 ± 10	6.3 ± 0.1	12 ± 1	74 ± 11
	Siliquae	368 ± 36	7.3 ± 0.1	12 ± 0	53 ± 9
	Roots	1810 ± 102	7.8 ± 0.4	9.7 ± 0.3	104 ± 10

Results indicate that there were no significant differences between varieties (spring and winter varieties) in terms of the metals content. The fractions of the biomass that showed a higher accumulation were the leaves and siliquae. The major element is K, which reflects a common feature among energy and oil crops. In a decreasing order, the biomass accumulates Ca, Mg, Na, Fe, Zn, Cu and Mn is the element presenting the lowest concentration.

Putnik-Delić *et al*, (2013) also verified higher Fe accumulation values in leaves compared to other fractions of biomass. In relation to Cu accumulation, the results obtained by Putnik-Delić *et al*, (2013) were higher than those recorded in the two varieties of *Camelina sativa*. In contrast, both varieties of C. *sativa* have higher values for Mn and Zn content in leaves and stems.

Nevertheless, it's difficult to compare the results obtained with data from literature. There is a lack of information on the metal content of the different fractions of *C. sativa*. Moreover, some literature presents data from the cakes obtained, and the relation with our data is also difficult to establish. Yet, it's possible that the same varieties obtained in different soils and climatic conditions could show different metals content as it was indicated in the study of Matthaus *et al*. (2000).

8. Conclusions

The use of plants that have phytoremediation capacity is a technology that has as principle the removal or reduction of concentration of contaminants, for example, soils contaminated by heavy metals or contaminated wastewaters. This is a sustainable, green technology that is in increasing use and has been subject of several studies in recent years.

Some studies had already proved the phytoremediation ability of *Camelina sativa* to heavy metals, for example. Thus, this study aimed to understand if both varieties of *C. sativa* (winter and spring variety) had phytodepuration capacity to an effluent obtained through the HTC process and that is highly pollutant. From the analysis conducted in this study, it´s possible to verify that the soil-biomass system was able to depurate the HTC effluent, avoiding the groundwater contamination. This suggest that, *Camelina sativa* has phytodepuration capacity for the HTC effluent.

The analysis of percolated waters allowed to conclude that the soil-biomass system was able to efficiently depurate the HTC effluent. Namely, analysis of total phenols, total acidity and oxidability demonstrated that the soil-biomass system was able to decrease the initial values of the HTC effluent. Nevertheless, the soil system was also able to depurate the contaminants present in the HTC effluent as evidenced by the analysis of the percolated waters and the GC-MS analysis. In GC-MS analysis, it was concluded that the two soil-biomass systems reduced the contaminants detected in the HTC effluent. Therefore, further studies are needed to understand the role of the soil in the phytodepuration process.

Concerning the characterization of biomass, in general it´s possible to conclude that HTC effluent irrigation (WW1, WW2 and WW3) didn´t significantly affect the steams height. However, the aboveground yield of the two varieties of *Camelina sativa* were affected by HTC effluent, as they present lower values than the control. Likewise, siliquae yield decreased especially when the spring variety was irrigated with WW3. These results may have been influenced by the restricted space delimited by the pot, which prevented a greater growth of the plant root system, which consequently limited the aboveground and siliquae yield. The high temperatures to which the plants were subjected at the end of this study, may also have contributed to the development of a state of senescence, and consequent decrease of yield values. But this effect was similar to plants irrigated with WW and control plants.

The chemical parameters analysis allowed to conclude that the plant´s structure that accumulated the most ash content was the leaves, followed by siliquae. The high percentage of ash in this structure may have negative implications on the quality of *Camelina sativa* cake and on the oil content extraction, affecting the exploitation of those seeds for biodiesel. Regarding the nitrogen, phosphorus and metals content it was verified that *C. sativa* showed more accumulation, particularly in the leaves and siliquae. But, generally, the application of HTC effluents did not affect significantly the biomass characteristics in terms of these parameters (N, P and metals).

In conclusion, the winter variety of *Camelina sativa* presents to be the most promising variety for phytodepuration. This variety showed greater resistance to the different cultivation conditions over time, namely to the WWs irrigation, as well as, greater resistance to environmental conditions, particularly high temperatures at the end of the study. Moreover, the winter variety presented better yield results, namely siliquae yield compared to the spring variety. In addition to that, it also had a lower ash content compared to the spring variety which suggests that this variety may be used as biomass for energy purposes.

9.Future Work

Following the research carried out for this master's thesis, it will be interesting to study in more detail some topics that weren´t addressed in the course of this study. In this way, it will be interesting to analyze the soil, to identify if soil characteristics were modified due to the application of those effluents. In addition, as this study is one of the few that analysis the phytodepuration capacity of *Camelina sativa*, it would be interesting to study its ability to depurate other contaminated effluents, as well as, to study its scale-up on the field. As the amount of biomass was small, it was difficult to characterize the seeds in terms of oil content and in terms of the fatty acid composition of the oil. It's important in a phytodepuration essay, to evaluate not only the remediation capacity of the biomass to the effluents but also to identify if the biomass being produced changes composition due to the characteristics of the effluents.

Finally, it´s also suggested to evaluate the potential of *Camelina sativa* in the concept of biorefinery. This evaluation should address the environmental, economic and social life cycle of the process optional chains, from the production of the seeds to its processing, use and disposal.

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Appendices

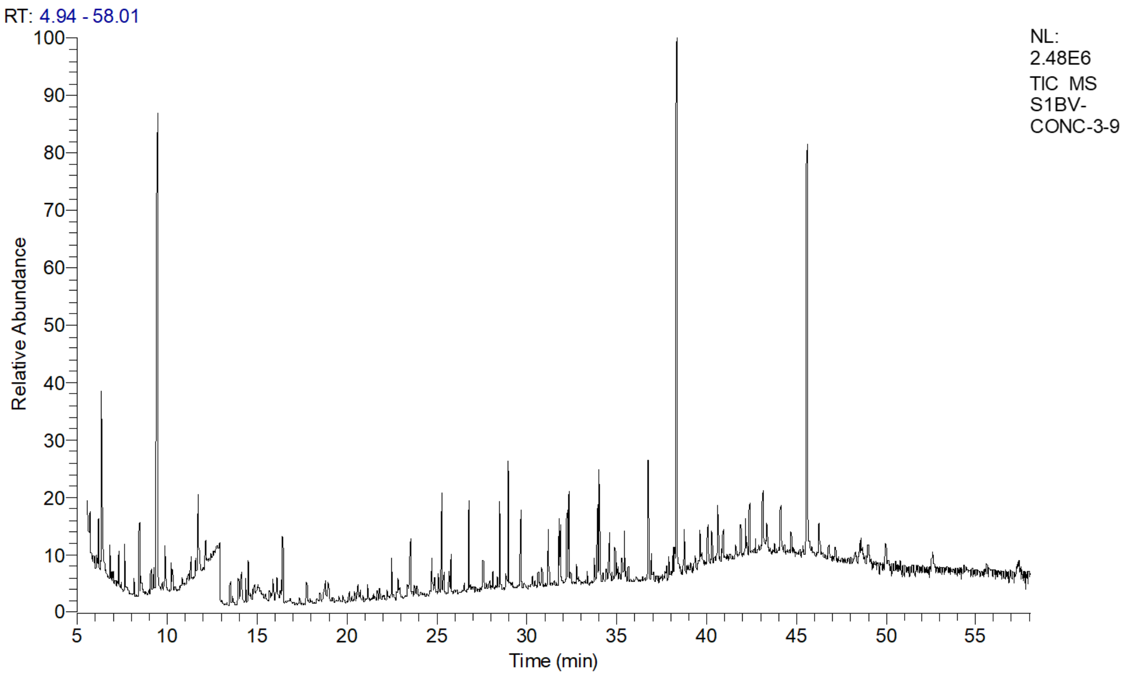


Figure A.1- Chromatographic profile of the organic compounds detected in the percolated water (WW1) of January in a soil- system.

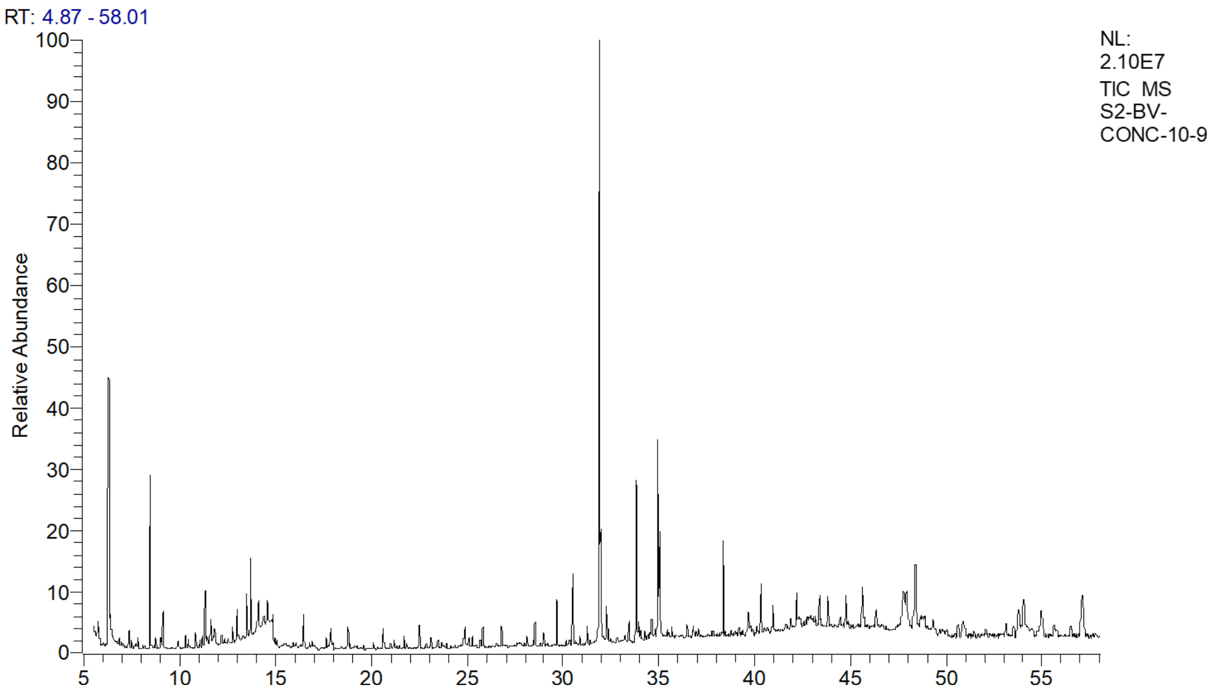
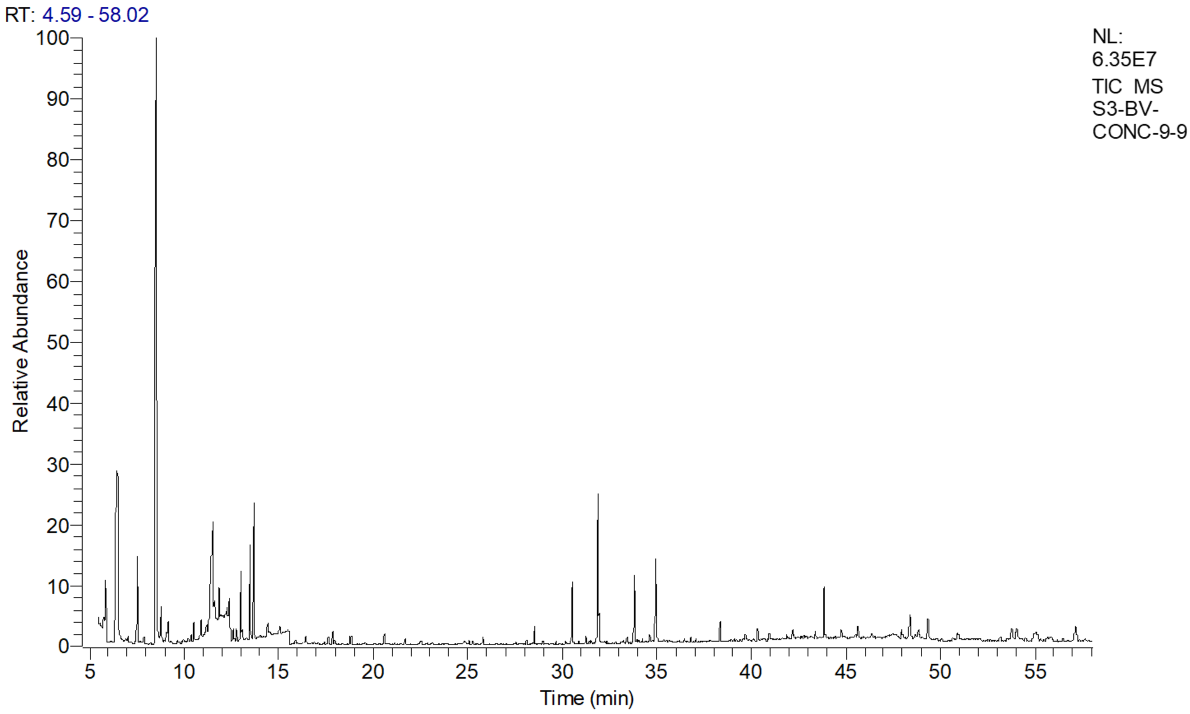


Figure A.2- Chromatographic profile of the organic compounds detected in the percolated water (WW2) of January in a soil- system.



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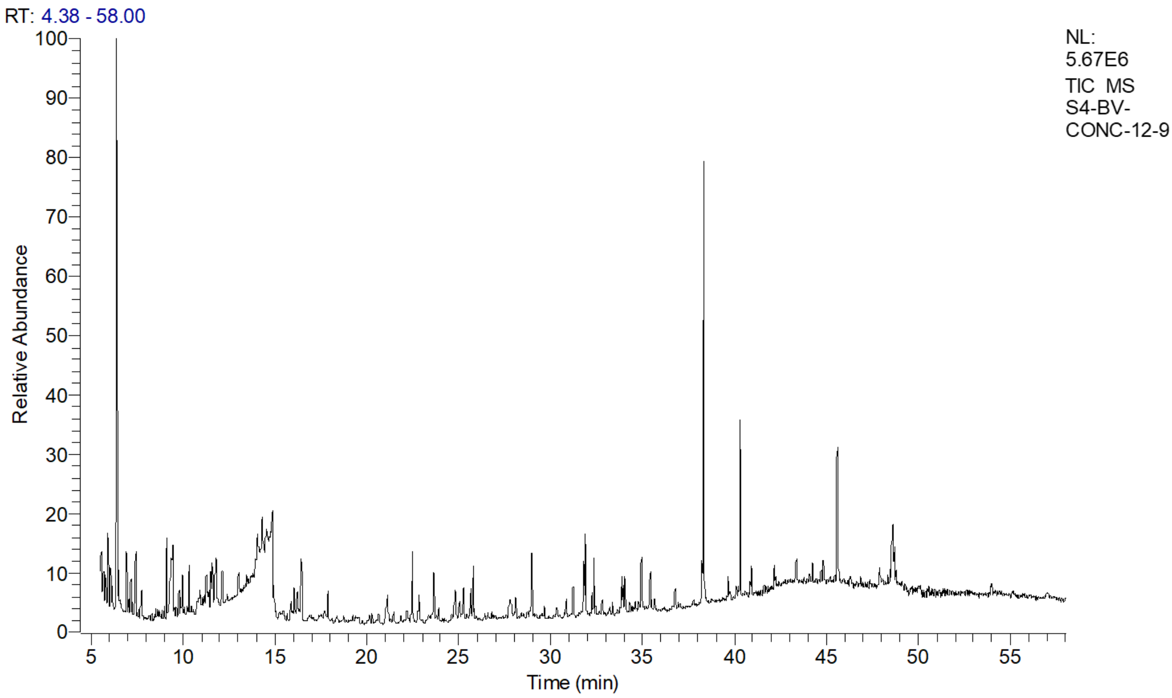

Figure A.4- Chromatographic profile of the organic compounds detected in the percolated water (control) of May in a soil- system.

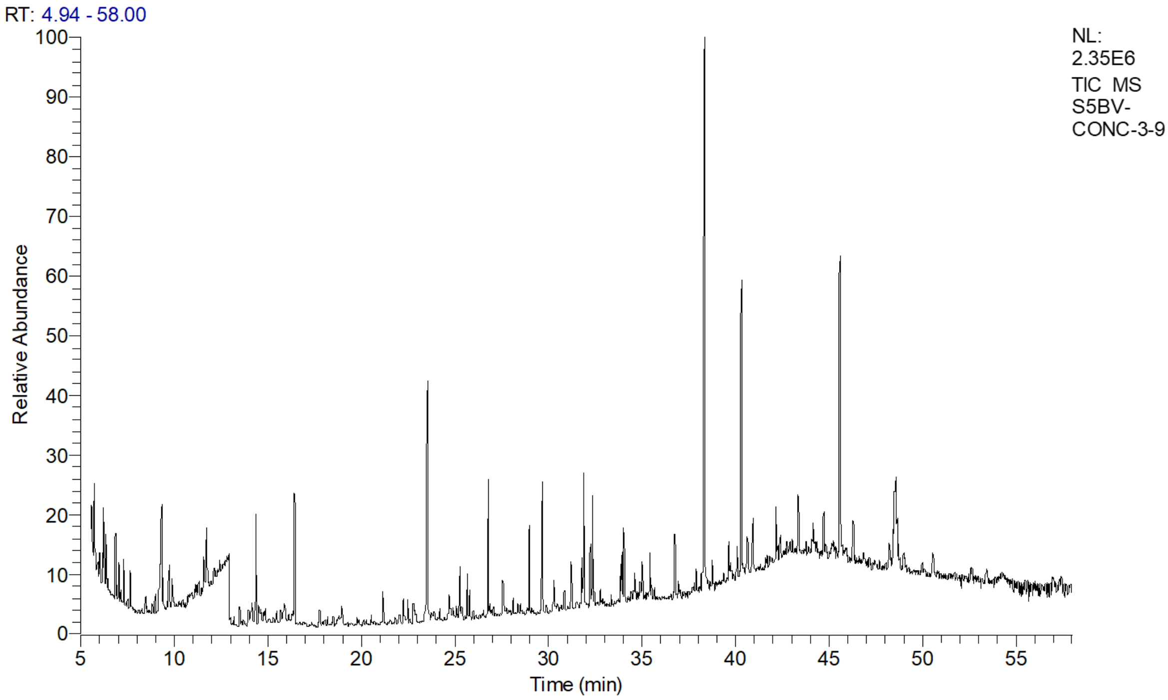

Figure A.5- Chromatographic profile of the organic compounds detected in the percolated water (WW1) of May in a soil- system.

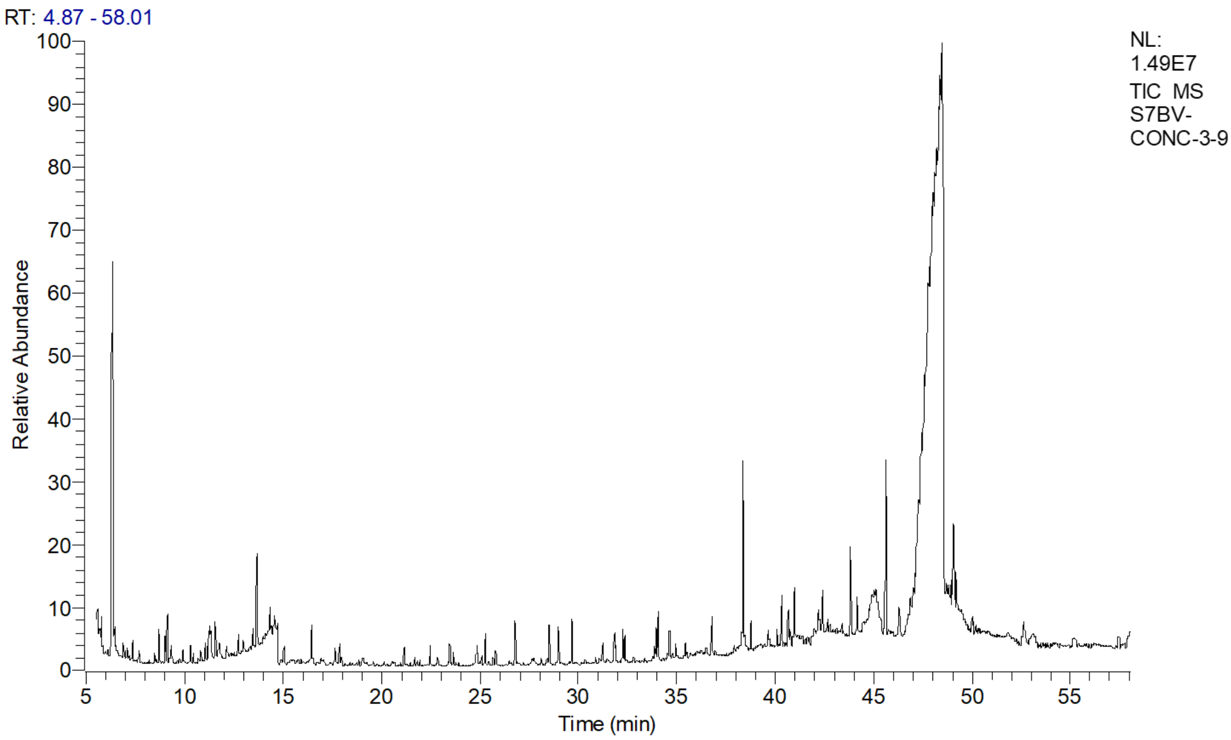

Figure A.6- Chromatographic profile of the organic compounds detected in the percolated water (WW3) of May in a soil- system.

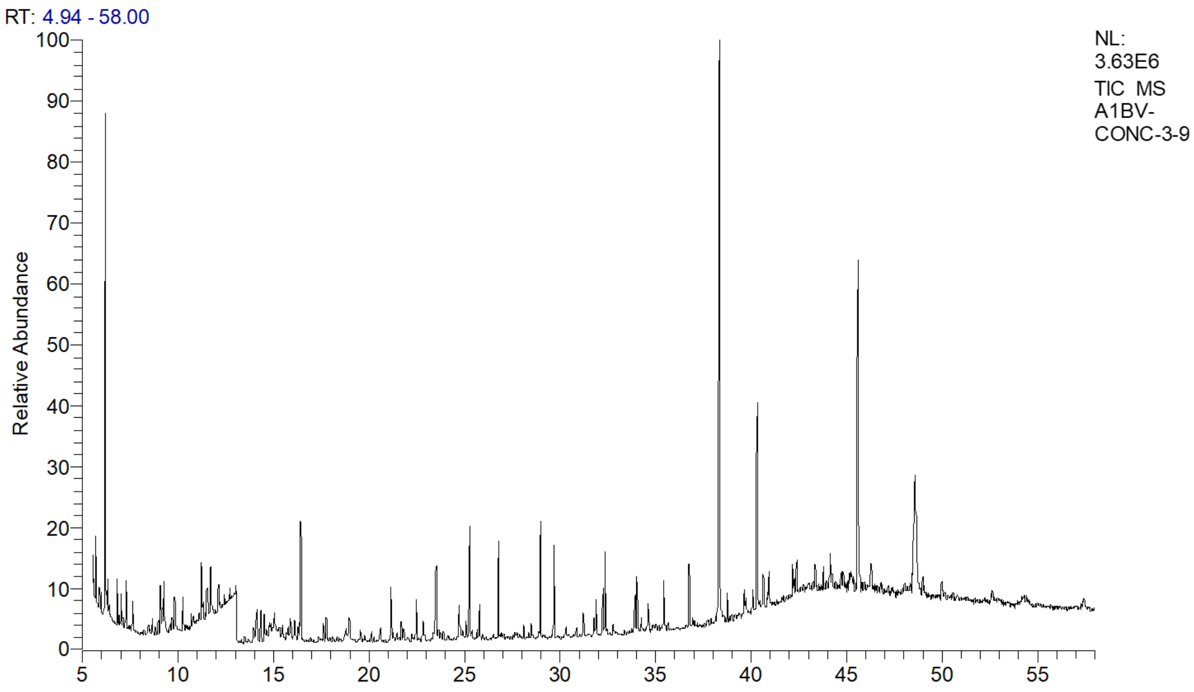

Figure A.7- Chromatographic profile of the organic compounds detected in the percolated water (control) of May in a soil- biomass system (*C.sativa*, winter).

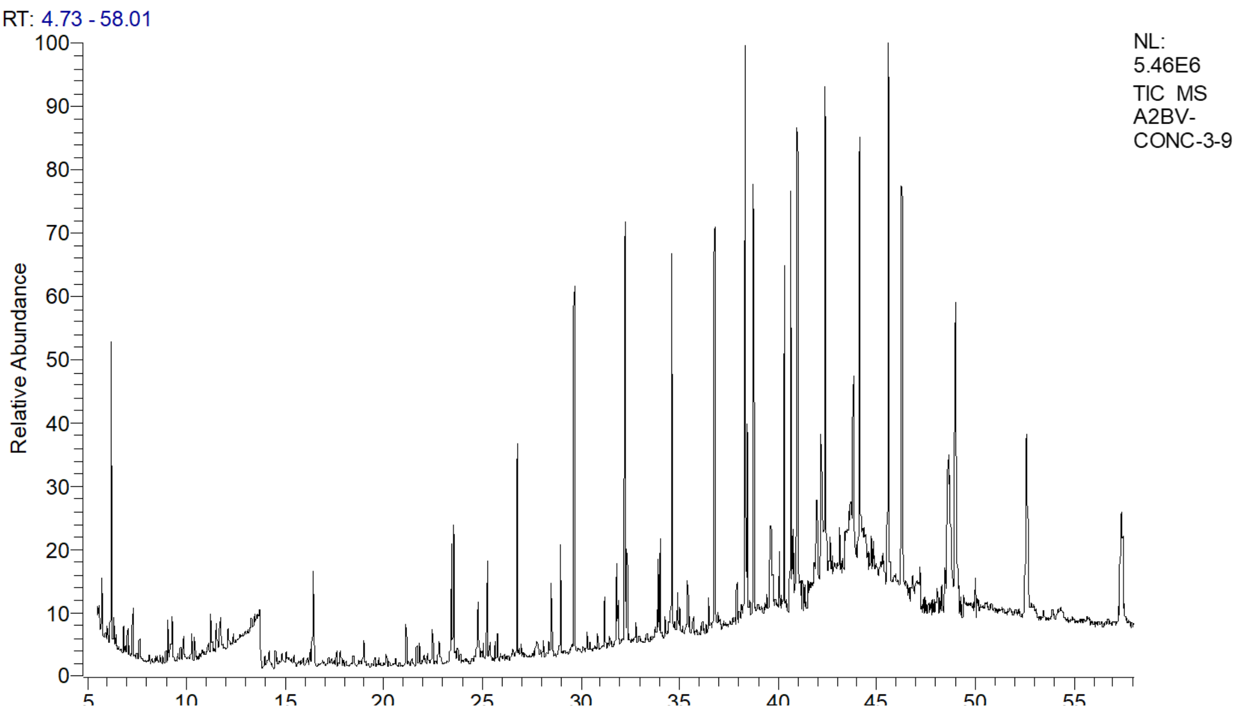

Figure A.8- Chromatographic profile of the organic compounds detected in the percolated water (control) of May in a soil- biomass system (*C.sativa*, spring).

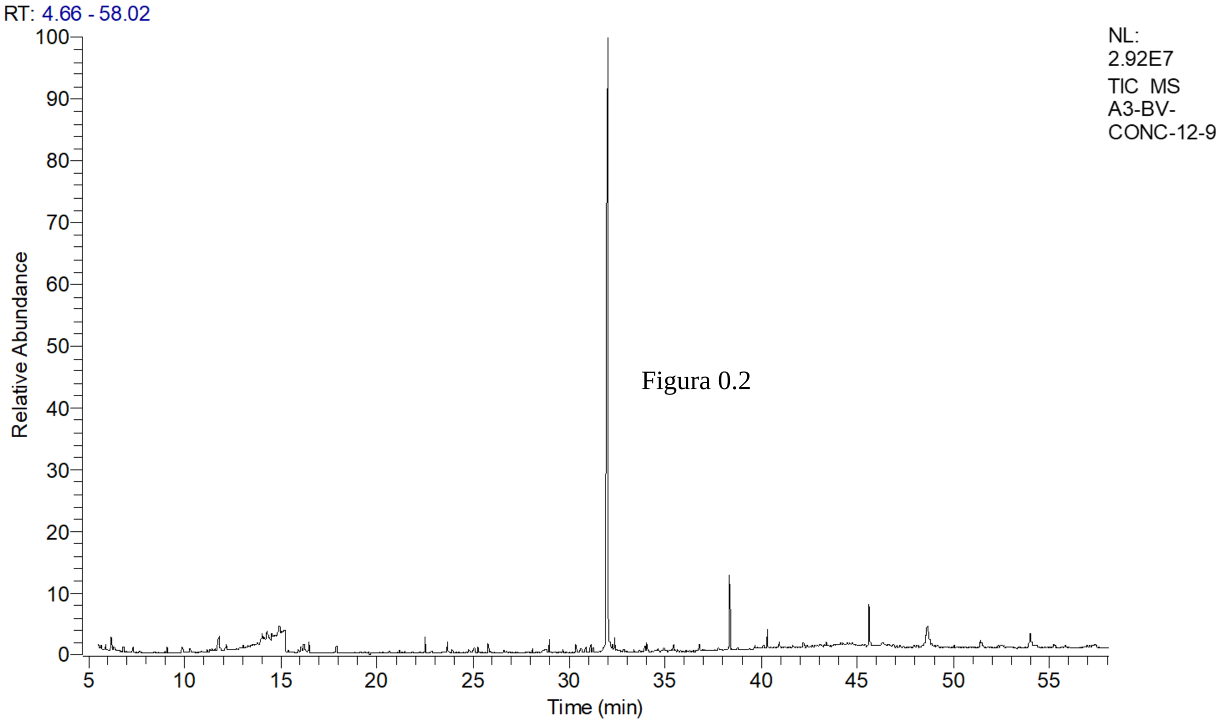

Figura 0.2

Figure A.9- Chromatographic profile of the organic compounds detected in the percolated water (WW1) of May in a soil- biomass system (*C.sativa*, winter).

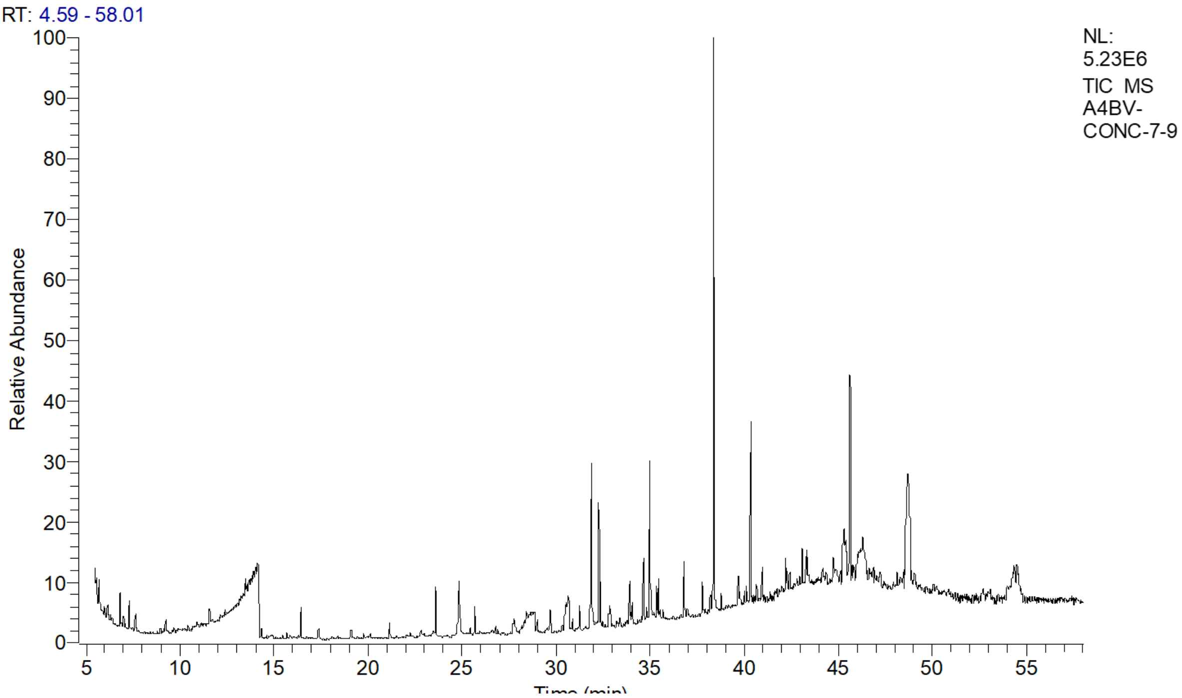

Figure A.10- Chromatographic profile of the organic compounds detected in the percolated water (WW1) of May in a soil- biomass system (*C. sativa*,spring).

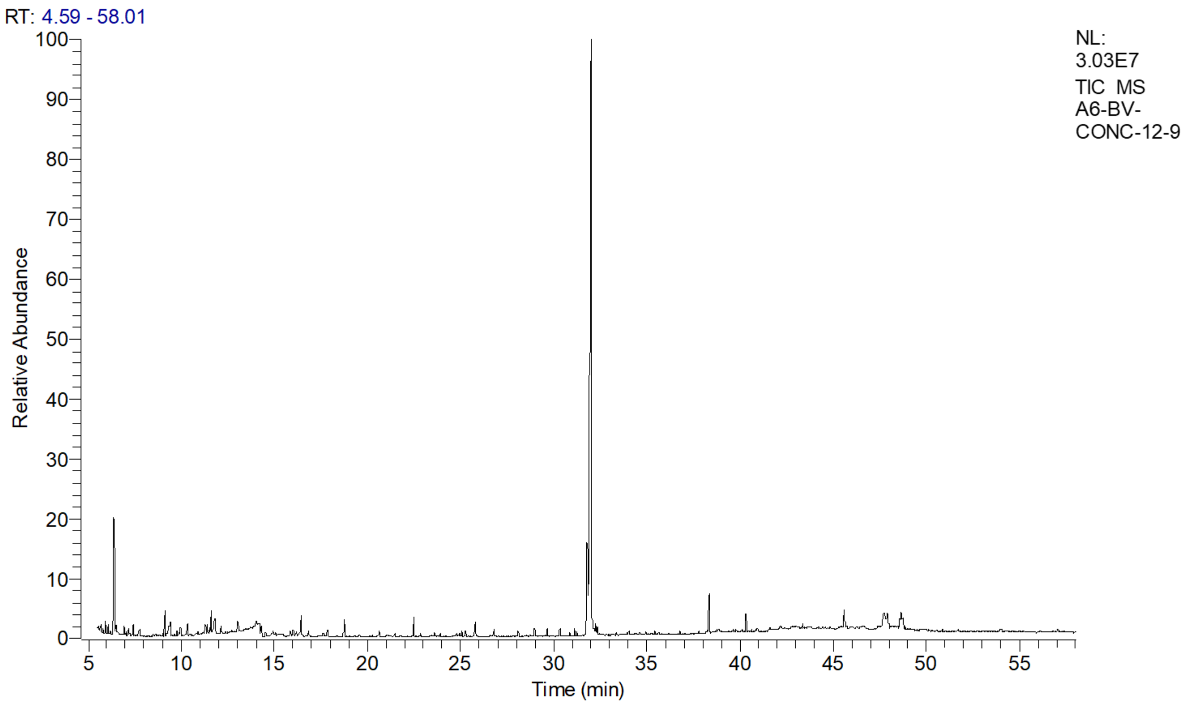

Figure A.11- Chromatographic profile of the organic compounds detected in the percolated water (WW2) of May in a soil- biomass system (*C.sativa*,spring).

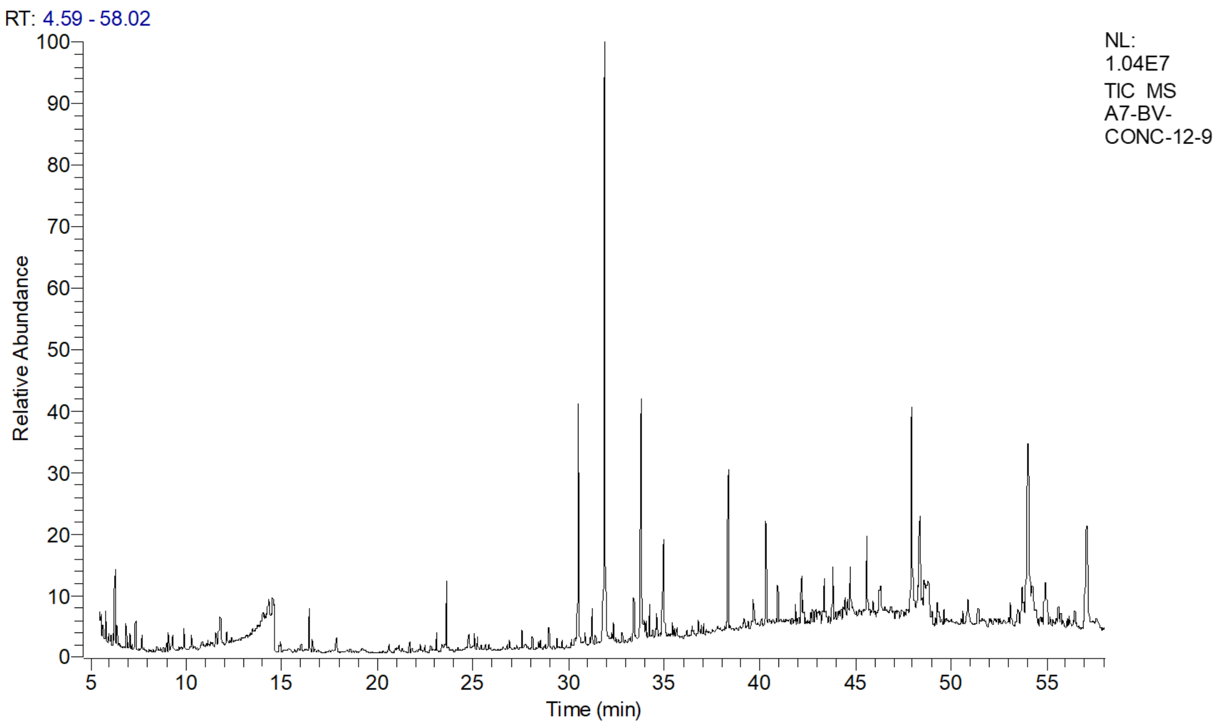

Figure A.12- Chromatographic profile of the organic compounds detected in the percolated water (WW3) of May in a soil- biomass system (*C.sativa*, winter).

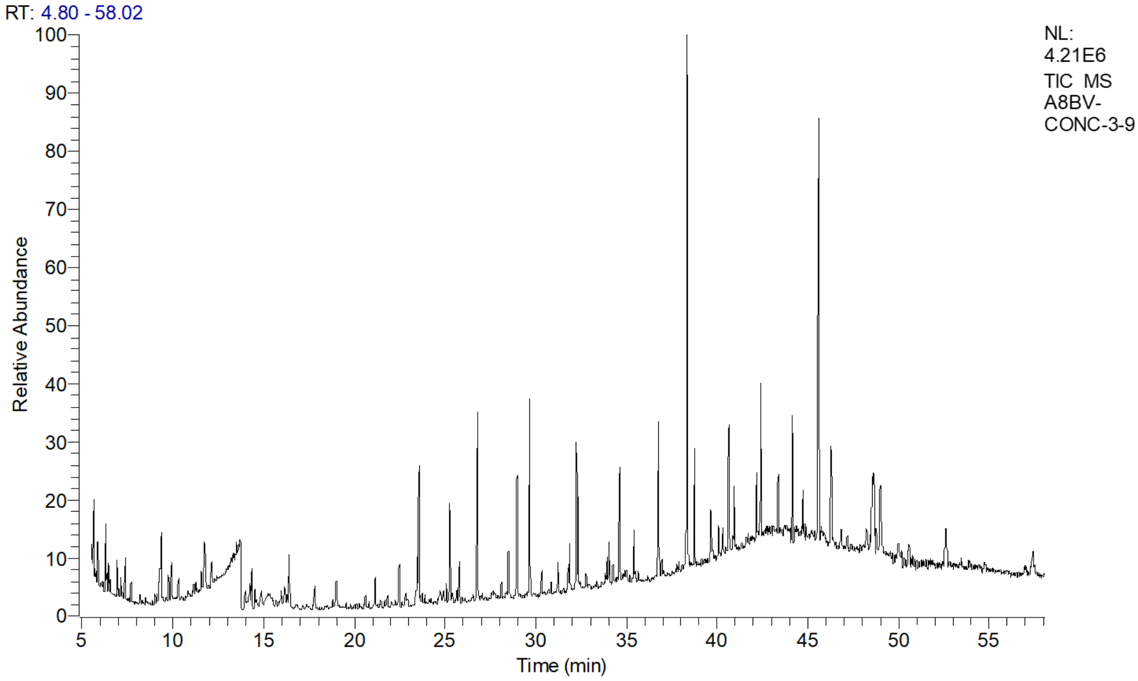

Figure A.13 - Chromatographic profile of the organic compounds detected in the percolated water (WW3) of May in a soil- biomass system (*C.sativa*,spring).